COMBINED WORK/QUALITY ASSURANCE PROJECT PLAN (CW/QAPP)

for

WATER COLUMN MONITORING 1998 – 2000
Tasks 9, 10, 12, 13, 14, 15

MWRA Harbor and Outfall Monitoring Project

submitted to

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MWRA Harbor and Outfall Monitoring Project
Water Column Monitoring 1998 – 2000
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2.0 PROJECT REQUESTED BY

Massachusetts Water Resources Authority
Environmental Quality Department

3.0 DATE OF REQUEST

November 5, 1997

4.0 DATE OF PROJECT INITIATION

November 5, 1997

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7.0 PROJECT DESCRIPTION

The Massachusetts Water Resources Authority (MWRA) is responsible for the operation and monitoring of the new sewage effluent outfall (Figure 1) from the Deer Island Wastewater Treatment Plant, which is scheduled to begin discharging in late 1998. The outfall is regulated under a National Pollutant Discharge Elimination System (NPDES) permit issued by the U.S. Environmental Protection Agency (EPA) and the Massachusetts Department of Environmental Protection (EPA/MDEP 1998). The EPA Supplemental Environmental Impact Statement (SEIS) for the outfall (EPA 1988) determined that there would be no significant water quality or biological impacts associated with the new outfall. The SEIS requires monitoring for compliance with the NPDES permit, assessing unacceptable impacts, and collecting data useful for outfall management considerations (MWRA 1990). MWRA is committed to implementing "long-term biological and chemical monitoring to describe existing conditions and evaluate the impacts of the treatment facility discharge." Public, scientific, and regulatory areas of concern were identified following guidance for coastal monitoring (*i.e.*, NRC 1990), and include water column, benthos, and fish and shellfish environments in addition to the effluent characteristics. The resulting MWRA monitoring program is designed to gather baseline environmental data, assess potential environmental impact of effluent discharge into Massachusetts Bay, and evaluate compliance with the discharge permit.

A principal concern with the offshore outfall discharge is nutrients and their resultant eutrophication effects on the water column. Three specific effects are of paramount concern: (1) lowered DO concentrations (hypoxia/anoxia), (2) stimulation of nuisance/noxious algae populations, and (3) alteration of the offshore food web. Water quality monitoring centers on measurements keyed to these three principal ecological effects, including measurements of other physical and chemical properties. For example, temperature, salinity, and turbidity are used to distinguish water masses and are fundamental background data for interpreting biological fluctuations. Physical features such as thermal stratification strongly influence the expression of nutrient enrichment effects. Measured nutrient concentrations (particulate and dissolved forms) aid water mass analyses, assess biological variability in light of nutrient variability, and, ultimately, link cause (nutrient loading) and effect. Zooplankton community monitoring will measure the pelagic food web and, in tandem with physical and chemical factors, may explain changes in the phytoplankton community.

7.1 Objectives and Scope

The primary objective of the MWRA Water Column Monitoring Program is *to detect changes in the physical water properties, nutrient concentrations, dissolved oxygen, phytoplankton biomass, and phytoplankton and zooplankton community composition in Massachusetts Bay and Cape Cod Bay*. These data will be used to evaluate MWRA Contingency Plan (MWRA 1997a) and Outfall Monitoring Plan (MWRA 1997b) caution and warning trigger parameters.

This Combined Work/Quality Assurance Project Plan (CW/QAPP) describes the sampling and analysis activities of MWRA's water column monitoring program beginning in 1998 and continuing through 2000 (MWRA Contract S274) and is based largely on CW/QAPPs of previous MWRA monitoring programs (Albro *et al.*1993; Bowen *et al.* 1998). Several water column surveys will be conducted to monitor water properties, nutrient concentrations, and other parameters that measure eutrophication, and to gain a better understanding of the physical processes that will affect the outfall plume.

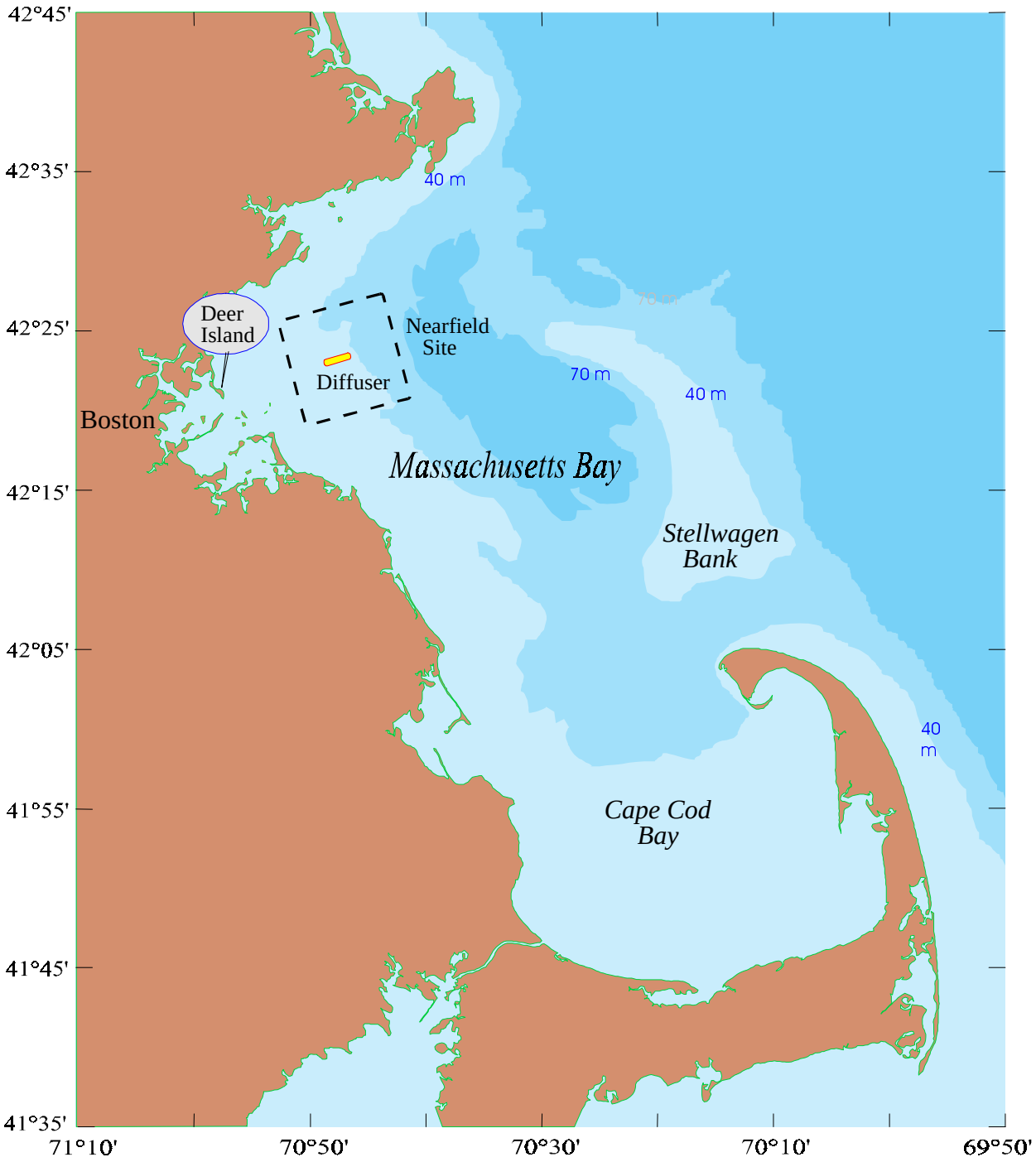


Figure 1. Location of MWRA Effluent Outfall in Massachusetts Bay

Eleven nearfield and six combined nearfield/farfield water column surveys of Massachusetts Bay and Cape Cod Bay will be conducted yearly beginning in 1998 and continuing through 2000. Physical and meteorological data collected by stationary moorings and satellites will supplement data collected during the water column surveys. Water quality, nutrients, and metabolism will be measured, and the phytoplankton and zooplankton communities will be described. The study objectives are described below.

- Develop a three-dimensional picture of seasonal variability of water column properties in the nearfield; determine the status of dissolved oxygen prior to the autumnal overturn in Stellwagen Bank; and determine the maximum winter concentration of dissolved inorganic nitrogen in Massachusetts Bay. (Task 9: Nearfield Surveys)

- Determine conditions in the water column throughout Massachusetts and Cape Cod Bays; identify factors affecting the seasonal pattern of plankton communities and dissolved oxygen concentrations in Massachusetts Bay); describe the broad-scale interaction of water from Boston Harbor and the Gulf of Maine with Massachusetts Bay; and compare water quality of Massachusetts and Cape Cod Bays. (Task 10: Farfield Surveys)

- Use data from two U.S. Geological Survey (USGS) moorings (one near the outfall site and one offshore Scituate) to interpolate temperature, salinity, chlorophyll a, and beam transmittance data between surveys, and data from meteorological stations to support explanations of environmental patterns observed in other tasks. (Task 12: Moorings and Meteorology)

- Acquire a synoptic view of water-column properties by using satellite remote sensing data to complement survey data. (Task 13: Remote Sensing)

- Describe the water quality by measuring concentrations of dissolved inorganic nutrients (nitrate, nitrite, ammonium, phosphate, and silicate), dissolved organic nitrogen and phosphorous, dissolved organic carbon, particulate carbon and nitrogen, particulate phosphorous, biogenic silica, chlorophyll *a* and phaeopigments, total suspended solids (TSS), dissolved oxygen (DO), urea, respiration, and primary productivity. (Task 14: Water Chemistry and Metabolism)

- Characterize the phytoplankton and zooplankton communities and describe subtle changes in community structure. (Task 15: Plankton Taxonomy)

The results of the sampling and analytical tasks will be reported in survey reports, data reports, synthesis reports, and other interpretive reports. Task 11 Plume Tracking Surveys will be described in a separate CW/QAPP to be completed in mid-1998 prior to commencement of the surveys.

7.2 Data Usage

Under the monitoring approach developed and adopted by MWRA and the Outfall Monitoring Task Force (OMTF)[1], public, scientific, and regulatory areas of concern were identified following NRC (1990) guidance for coastal monitoring. Using this information, a draft Phase I Baseline Monitoring Plan (MWRA 1991) was developed, reviewed, and accepted by EOEA with revisions (Pederson 1992). This plan described and discussed the ecological and other potential responses (perturbations) that were of concern ("trigger parameters") and the field and laboratory studies that were necessary to acquire data to

[1] The OMTF was established by the Massachusetts Executive Office of Environmental Affairs (EOEA) to oversee the monitoring program.

address these concerns. Details of the field and analytical program conducted under Phase I are described in a series of Combined Work/Quality Assurance Project Plans (Butler *et al.* 1995; Blake and Hilbig 1995; Mitchel *et al.* 1995) with subsequent program revisions as data became available and in response to other recommendations (Hunt and Steinhauer 1994a,b; Hunt *et al.* 1994; McCarthy *et al.* 1996a,b,c). Delays in the completion of the discharge into Massachusetts Bay has allowed the collection of six years (1992 to 1998) of baseline data rather than the original three years required.

The Phase 2 Post-Discharge Monitoring Plan (MWRA 1997b) (hereafter referred to as the Monitoring Plan) focuses on the environment in the vicinity of the future outfall, with additional effort in Cape Cod and Massachusetts Bay. Improvements in Boston Harbor are also monitored. The objectives of the Monitoring Plan are to (1) test for compliance with NPDES permit requirements, (2) verify that the impact of the discharge on the environment is within the bounds predicted by the EPA SEIS (with National Marine Fisheries Service concurrence); that is, no significant water quality or biological impacts are associated with the outfall; and (3) test whether change within the system exceeds the Contingency Plan thresholds.

The Monitoring Plan is complemented by two documents: the Outfall Monitoring Overview, which describes the results of studies implemented under the Monitoring Plan, and the Contingency Plan, (MWRA 1997a) which lists thresholds (Caution and Warning Levels; Table 1) developed to protect the environment and public health.

Table 1. Water Column Trigger Parameters and Thresholds[a]

Parameters	Caution Level	Warning Level
Dissolved oxygen concentration (nearfield bottom and Stellwagen bottom)	6.5 mg/L for any one month during stratification[b]	6 mg/L, 75% saturation for any one month during stratification[b]
Dissolved oxygen depletion rate (nearfield bottom)	1.5 X baseline for any one month during stratification[b]	2 X baseline for any one month during stratification[b]
Chlorophyll (nearfield)	1.5 X baseline annual mean 95th percentile of the baseline seasonal distribution	2 X baseline annual mean --
Nuisance and noxious algae (nearfield)	95th percentile of the baseline seasonal mean	--
Zooplankton (nearfield)	Shift toward inshore community	--
Diffuser mixing		--
Paralytic shellfish poisoning (PSP) (farfield)	New incidence	--

[a]From the Contingency Plan (MWRA 1997a).
[b]Stratification months are considered June through October.

The Contingency Plan thresholds are based on expected permit limits, observations from the baseline monitoring, national water quality criteria and state standards, and in some cases, best professional judgment. In the event that one of these thresholds is exceeded, the Contingency Plan sets into motion an environmental management process to (1) confirm the threshold exceedance; (2) determine the causes and significance of the exceedance; and, if the environmental changes are attributable to the effluent outfall, (3) identify the actions that will be taken to return the trigger parameter to an acceptable level. Examples

of management actions include additional monitoring, development of response plans and performances of engineering feasibility studies.

The baseline monitoring has shown fairly large variations in the measured parameters, as is expected in complex environmental systems. The statistical power of detecting change has been treated at various times by Hunt *et al.* (1995) for water column, and also by Hunt and Baptiste (1993) for fish and shellfish and Coats (1995) for sediment chemistry. In general, detectable change can be as low as 10 to 20% for dissolved oxygen; 50 to 100% for fish and shellfish parameters and some sediment chemicals; and 100 to 200% for chlorophyll and dissolved inorganic nitrogen. The proposed Monitoring Plan is expected to provide a high probability (80%) of detecting appreciable change. Many of the Contingency Plan thresholds are greater than current baseline conditions (*e.g.* mercury levels in fish) such that statistically significant changes would be detected long before the threshold is approached.

7.3 Technical Approach

The study consists of sampling surveys and analysis of samples collected during those surveys. The technical approach to completing those tasks is discussed below.

7.3.1 Nearfield and Farfield Water Column Surveys (Tasks 9 and 10)

Water column sampling will be conducted 17 times per year in 1998, 1999, and 2000 (Figure 2). Figure 3 shows the location of the 21 nearfield stations (Table 2) and 26 farfield stations (Table 3) that will be sampled each year.

7.3.1.1 Sampling Locations and Frequency

Nearfield stations are located within the area defined to encompass the expected 2-day excursion of the outfall discharge, and are placed to characterize (1) the varying path of the discharge plume, (2) the plume-pycnocline interaction, and (3) the influence of Boston Harbor and northern rivers. Each nearfield sampling will be completed in one day. Three station types (A, P, and E) are sampled in the nearfield. Table 4 shows subsampling by station type and sample depth. Stations N04 and N18 will be sampled early in the day to allow time for the photosynthesis (productivity) incubations and to avoid disturbing the diurnal plankton cycles.

Farfield stations are located beyond the nearfield to (1) cover regional-scale oceanographic processes in Massachusetts Bay and Cape Cod Bay; (2) broadly characterize reference areas; and (3) to verify that impacts by the outfall plume are not found beyond the nearfield. Each farfield sampling will be completed in three or four days. However, during the first three farfield surveys each year, two additional stations (F32 and F33) will be profiled for hydrographic data and sampled for zooplankton. The six farfield surveys conducted each will capture the conditions at six times or seasons: winter (mid-February), late winter, early spring (early March), spring (early April), early summer (mid-June), late summer (mid-August), and early fall (mid-October). Each year, all six farfield surveys will be combined with one-day nearfield surveys.

7.3.1.2 Combined Surveys

Six of the 17 one-day nearfield surveys will be combined with the three-day farfield surveys. During these combined four-day surveys, farfield operations will be started prior to the nearfield operations to optimize mobilization and total transit time. The farfield areal productivity station (F23) will be sampled on the nearfield day to confine all productivity processing to one survey day. The farfield stations will be completed on the last day.

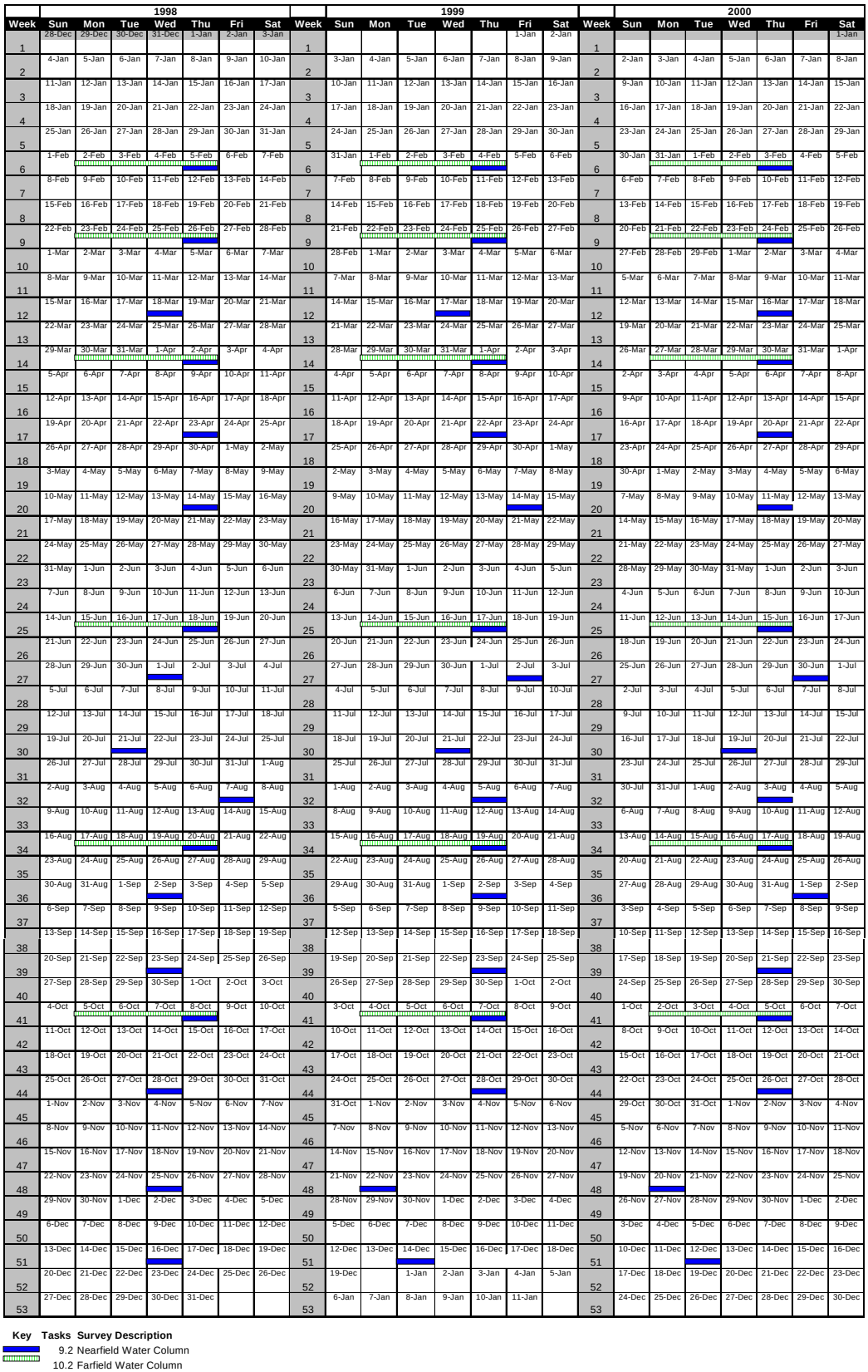


Figure 2. Water Column Sampling Schedule, 1998-2000

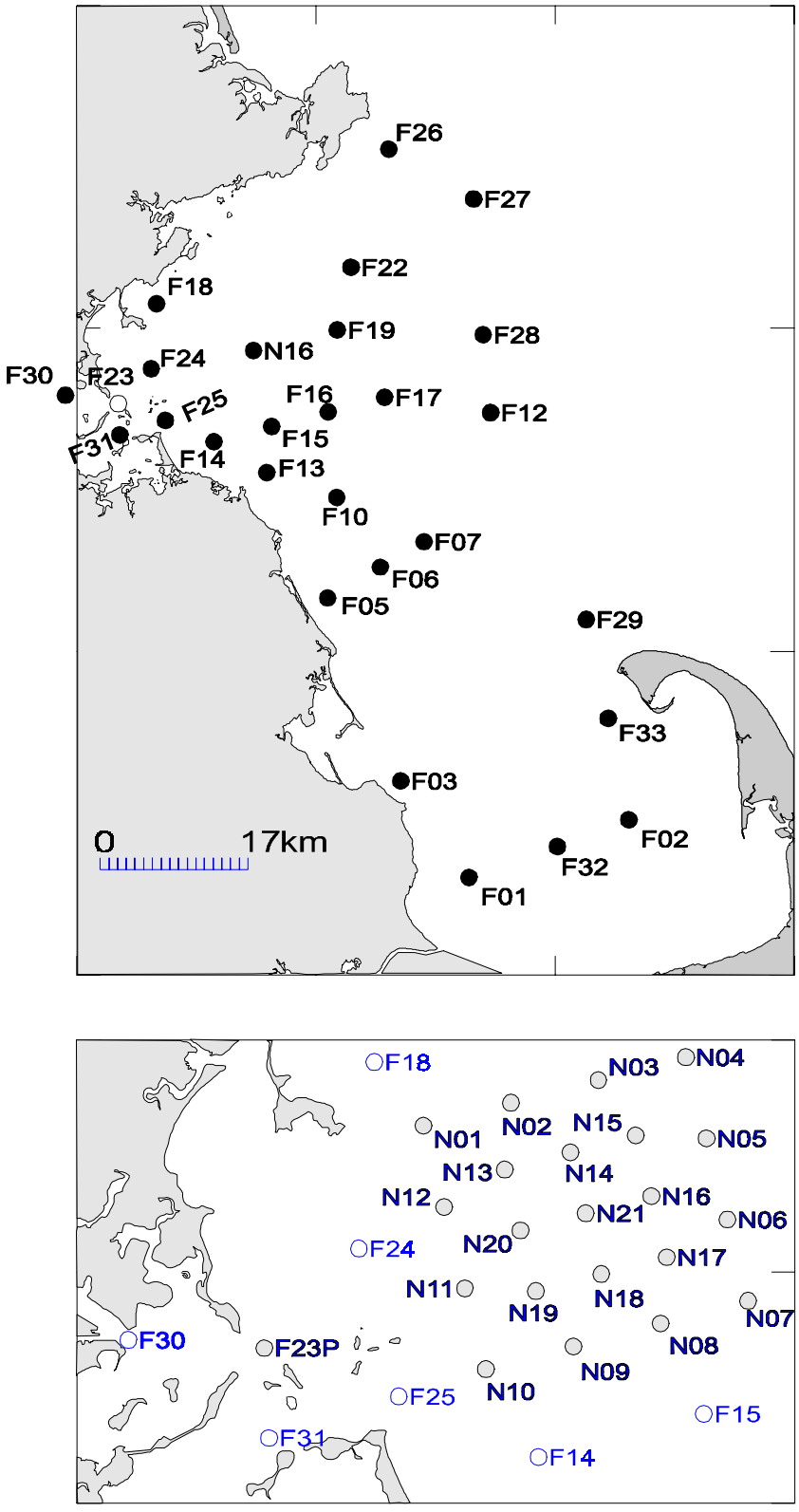


Figure 3. Sampling Stations during Combined Farfield (Days 1, 2, and 4) and Nearfield (Day 3) Water Column Surveys

Table 2. Nearfield Water Column Sampling Stations

Station	Latitude (N)	Longitude (W)	Depth (m)	Station Type
N01	42° 25.16'	70° 51.87'	30	A
N02	42° 25.65'	70° 49.31'	40	E
N03	42° 26.14'	70° 46.75'	44	E
N04	42° 26.63'	70° 44.19'	50	P
N05	42° 24.88'	70° 43.58'	55	E
N06	42° 23.13'	70° 42.97'	52	E
N07	42° 21.38'	70° 42.37'	52	A
N08	42° 20.88'	70° 44.93'	35	E
N09	42° 20.39'	70° 47.48'	32	E
N10	42° 19.89'	70° 50.04'	25	A
N11	42° 21.65'	70° 50.65'	32	E
N12	42° 23.40'	70° 51.26'	26	E
N13	42° 24.21'	70° 49.49'	32	E
N14	42° 24.58'	70° 47.57'	34	E
N15	42° 24.95'	70° 45.65'	42	E
N16	42° 23.64'	70° 45.20'	40	A
N17	42° 22.32'	70° 44.74'	36	E
N18	42° 21.95'	70° 46.66'	30	P
N19	42° 21.58'	70° 48.58'	24	E
N20	42° 22.90'	70° 49.03'	32	A
N21	42° 23.27'	70° 47.12'	34	E

Table 3. Farfield Water Column Stations

Station	Latitude (N)	Longitude (W)	Depth (m)	Station Type
F01	41° 51.05'	70° 27.20'	27	D
F02	41° 54.49'	70° 13.70'	33	D
F03	41° 57.00'	70° 32.90'	17	E
F05	42° 08.32'	70° 39.00'	18	E
F06	42° 10.24'	70° 34.60'	35	D
F07	42° 11.81'	70° 30.95'	54	E
F10	42° 14.54'	70° 38.24'	30	E
F12	42° 19.80'	70° 25.40'	90	F
F13	42° 16.10'	70° 44.10'	25	D
F14	42° 18.00'	70° 48.50'	20	E
F15	42° 18.93'	70° 43.66'	39	E
F16	42° 19.84'	70° 38.97'	60	E
F17	42° 20.75'	70° 34.23'	78	E
F18	42° 26.53'	70° 53.30'	24	E
F19	42° 24.90'	70° 38.20'	81	R
F22	42° 28.79'	70° 37.06'	80	E
F23	42° 20.35'	70° 56.52'	25	P
F24	42° 22.50'	70° 53.75'	20	D
F25	42° 19.30'	70° 52.58'	15	D
F26	42° 36.10'	70° 33.90'	56	E
F27	42° 33.00'	70° 26.84'	108	D
F28	42° 24.60'	70° 26.00'	33	E
F29	42° 07.00'	70° 17.40'	66	F
F30	42° 20.48'	71° 00.45'	15	G
F31	42° 18.38'	70° 56.40'	15	G
F32	41° 52.77'	70° 20.45'	15	Z
F33	42° 00.75'	70° 15.55'	15	Z
N16	42° 23.64'	70° 45.20'	40	D

Table 4. Subsamples by Station Type Code and Sample Depth Class

Subsample Analysis	Station Type Code[1]								Sample Depth Class[2]
	A	D	E	F	G	R	P	Z	
Dissolved Inorganic Nutrients	√	√	√	√	√	√	√		A, B, C, D, E
Dissolved Organic Carbon Total Dissolved Nitrogen Total Dissolved Phosphorous Particulate Organic Carbon Particulate Organic Nitrogen Particulate Phosphorous Biogenic Silica	√	√			√		√		A, C, E
Urea		√			√		√		A, C
Chlorophyll *a* and Phaeophytin *a*	√	√					√		A, B, C, D, E
					√				A, C, E
Total Suspended Solids	√	√			√		√		A, C, E
Dissolved Oxygen	√	√		√		√	√		A, B, C, D, E
					√				A, C, E
Zooplankton		√			√		√	√	Z
Phytoplankton (whole water)		√			√		√		A, C
Phytoplankton (screened water)		√			√		√		A, C
Respiration						√	√		A, C, E
Primary Productivity							√		A, B, C, D, E

[1]Defined by Suite of Analyses.
[2]Sample Depth Classes.
- A Surface (<3 meters)
- B Mid-surface
- C Mid-depth (chlorophyll *a* maximum)
- D Mid-bottom
- E Bottom (within 5 m of bottom)
- Z Upper 30 m tow through water column

7.3.1.3 Hydrocasts and Sensor Measurements

Hydrographic data will be collected at the nearfield stations and farfield stations. During the combined surveys, nearfield station N16 will be visited during both the nearfield and farfield survey days. At each station, a hydrocast will be conducted with an underwater unit consisting of a conductivity–temperature–depth (CTD) system, various sensors (dissolved oxygen, chlorophyll fluorescence, optical beam transmittance, light irradiance (PAR), and altimeter), and a water-sampling system equipped with up to 12 9–L Rosette sampling bottles.

Sensor measurements will be collected during the downcast from near surface to within approximately 3-5 m of the sea floor at each station. Salinity and Sigma-T will be calculated from the conductivity, temperature and depth data. Total incident photosynthetically available radiation at the surface (SPAR), navigational position, and time will be recorded concurrently with the hydrocast measurements.

7.3.1.4 Water Collection and Net Tows

During the upcast at each station (except stations F32 and F33), 9–L Rosette sampling bottles will be used to collect water from five depths: bottom, mid-bottom, middle (chlorophyll *a* maximum), mid-surface, and surface. At Boston Harbor stations F30 and F31, depths mid-bottom and mid-surface are not

sampled. On deck the Rosette sampling bottles will be subsampled for dissolved inorganic nutrients and other analyses as determined by the station type (Table 4). Vertical net tows will be conducted to collect zooplankton according to the scheme shown in Table 4.

7.3.1.5 Whale Observations

During each nearfield survey and the first three farfield surveys of each year, a trained whale observer will conduct sighting watches while on station and during transit between stations. The sighting operations will occur during daylight hours and when the vessel is in Massachusetts Bay or Cape Cod Bay. The observer will scan the ocean surface by eye for a minimum of 40 minutes every hour. All sightings will be recorded on standardized marine mammal field sighting logs (described in Section 12). Header fields for sighting logs will include observer name, time, date, weather, wind speed, sea state, vessel name, heading and speed. Data fields on sighting logs will include: vessel position every 5 minutes, time, observer position on vessel, sighting event code (on or off watch, transiting or on station), compass bearing to mammal, species name, number of animals, behavior, and sighting cue code.

7.3.1.6 Moorings and Meteorology (Task 12)

Physical oceanographic data collected by moored instruments operated by the U.S. Geological Survey (USGS) will be obtained under Task 12. Hydrographic data from stations near the mooring will be provided to the USGS. Meteorological data will be obtained from the National Weather Service weather stations at Deer Island, Logan Airport, and Provincetown Airport, which may be supplemented with data from other regional weather stations as necessary to complete the data set. Solar radiation data will be obtained from MWRA. In addition, electronic copies of Boston Harbor tide data from NOAA will be obtained, which will provide useful supporting information for the data obtained from near-shore stations and from the plume-tracking operations in and out of Boston Harbor.

7.3.1.7 Remote Sensing (Task 13)

Imagery Source. Sea surface temperature (SST) imagery is expected to be available in real-time and will be downloaded from the NOAA CoastWatch Northeast Node Internet site via the registered MWRA account. The NOAA CoastWatch Northeast Node utilizes internal QC procedures and validates the imagery with *in-situ* buoy data. The temperature derived from the satellite data has been shown to match the buoy data within 0.31 °C (Pichel *et al.* 1995).

Chlorophyll imagery from the SeaWiFS sensor will be similarly downloaded from the NOAA CoastWatch Northeast Node. The availability of the chlorophyll images, however, is expected to be delayed on the order of 2 to 3 weeks. The calibration and validation team on the SeaWiFS project has developed a data processing system with multiple layers of quality control. The SeaWiFS project utilizes data from vessels of opportunity and moorings to correlate the satellite data with *in situ* data. The primary mooring presently used in this process is the Marine Optical Buoy (MOBY) off the coast of Lanai, Hawaii. MOBY serves an important function as a calibration reference station for satellite instruments such as SeaWiFS, EOS COLOR, MODIS, MERIS, and ADEOS OCTS to assist in maintaining the accuracy of those instruments. In its role of measuring ocean color, MOBY provides a time-series database for bio-optical algorithm development. Because the buoy will acquire data 3-5 times per day at the same site, oceanographers can now monitor the daily fluctuations in biomass concentrations at that site and fine tune their algorithms accordingly.

Optimal Number of Images. SST and chlorophyll images will be obtained coincident with each day of the Nearfield and Farfield surveys. Assuming no atmospheric interference, the optimal number of images acquired each year will total 35. This number reflects the intended survey schedule of 6 Farfield surveys of 3 days each (18 days) and 17 Nearfield surveys of one day each.

Atmospheric Interference Alternatives. Clouds can be expected to cause significant atmospheric interference throughout the remote sensing task. Attempts will be made to acquire a satisfactory image for each day of the water column surveys. However, weather conditions may preclude the collection of satisfactory images within the time bracket of the survey. Therefore, if the skies are overcast during a survey, images will be collected according to the following levels of priority: (1) within 3 days prior to the survey; (2) within 3 days after the survey; (3) within 4 to 7 days prior to the survey; or (4) within 4 to 7 days after the survey.

7.3.1.8 Shipboard Sample Processing

Sample aliquots are removed from the Rosette sampling bottles and are processed aboard ship in preparation for shipment to the analytical laboratories. The water-sample-filtration scheme is detailed and graphically shown in Section 12.

7.3.2 Laboratory Program (Tasks 14, 15)

Water samples collected during the surveys will be analyzed to determine concentrations of dissolved inorganic nutrients (DIN) (nitrate, nitrite, ammonium, phosphate, and silicate); dissolved and particulate organic nutrients (carbon, nitrogen, and phosphorus); biogenic silica; urea; DO; TSS; chlorophyll *a* and phaeophytin; primary productivity, respiration rates, and phytoplankton and zooplankton community structure. The sample analyses are summarized in Table 5. Sampling and analytical methods are described in Section 12.

7.3.3 Data Management (Tasks 9 through 15)

Figure 4 illustrates the water-column-monitoring data processing to allow entry into the MWRA Environmental Monitoring and Management System (EM&MS) and to provide information to the public. The data from the program will be compared with the caution and warning trigger parameters in the MWRA Contingency Plan (MWRA 1997a) and Outfall Monitoring Plan (MWRA 1997b) to assess potential impacts.

7.4 Monitoring Parameters and Collection Frequency

Table 5 lists analytical parameters and *in situ* hydrographic measurements and Table 6 presents the collection frequency of each. Summary sampling plans for nearfield and farfield surveys are in Appendix A (Tables A1 and A2, respectively).

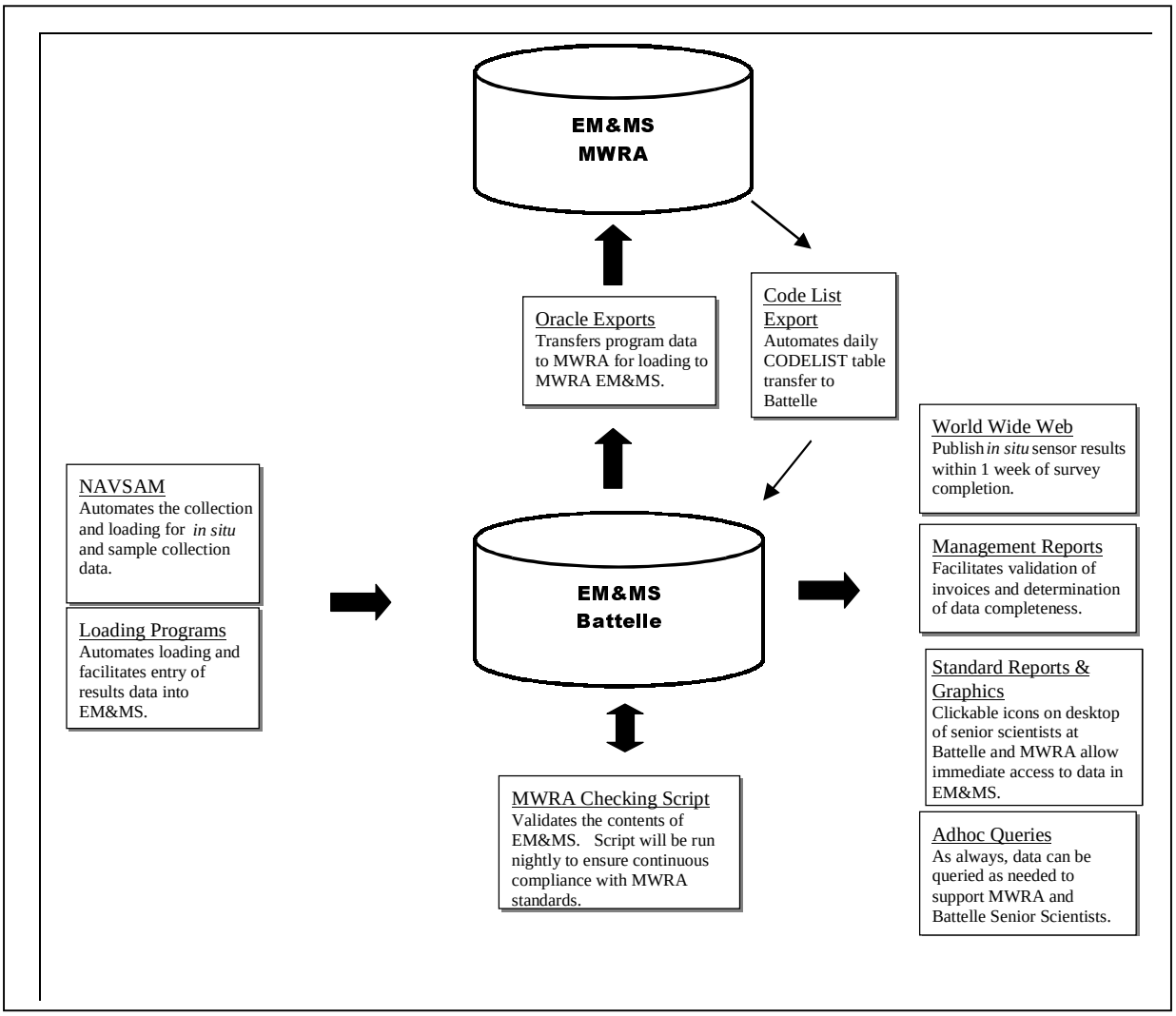


Figure 4. Overview of the Data Management Strategy for Water Column Monitoring

Table 5. Water Column Sample Analyses

Parameter	Lab	Units	Instrument	Reference
Dissolved ammonium	URI	µM	Technicon Autoanalyzer II	Lambert and Oviatt (1986); Solorzano (1969)
Dissolved inorganic nitrate and inorganic nitrite	URI	µM	Technicon Autoanalyzer II	Bendschneider and Robinson (1952), and Morris and Riley (1963)
Dissolved inorganic phosphorus	URI	µM	Technicon Autoanalyzer II	Murphy and Riley (1962)
Dissolved inorganic silicate	URI	µM	Technicon Autoanalyzer II	Brewer and Riley (1966); Lambert and Oviatt (1986)
Dissolved organic carbon	CBL	µM	Shimadzu 5000 Carbon Analyzer	Menzel and Vaccaro (1964)
Total dissolved nitrogen and total dissolved phosphorus	CBL	µM	Technicon Autoanalyzer II	D'Elia *et al.* (1997); Valderrama (1981)
Particulate carbon and particulate nitrogen	CBL	µM	Exeter Analyzer Model CE-440	EPA Method 440.0 (March 1997) Menzel and Vaccaro (1964)
Particulate phosphorus	URI	µM	Technicon Autoanalyzer II	Solorzano and Sharp (1980)
Biogenic Silica	CBL	µM	Technicon Autoanalyzer II	Paasche (1973)
Urea	MBL	µM	Shimadzu UV-Visible Spectrophotometer Model UV1601	Price and Harrison (1987)
Chlorophyll a/phaeopigments	Battelle	µg/L	Turner Designs Fluorometer Model 10AU	Arar and Collins (1992); Battelle SOP 5-265
Total suspended solids	Battelle	mg/L	Mettler 5-place balance	Battelle SOP 5-053
Dissolved oxygen	Battelle	mg/L	Radiometer TitraLab	Battelle SOP 5-257, Oudot *et al.*. (1988), and APHA *et al.* (1989)
Respiration	Battelle	µM/h	Radiometer TitraLab	Battelle SOP 5-257 and Strickland and Parsons (1972)
Primary production by ^{14}C	URI	$mgC/m^3/h$	Beckman LS3801 scintillation counter	Strickland and Parsons (1972); Lewis and Smith (1983)
Whole-water phytoplankton	UMD	E6Cells/L	Olympus BH-2 compound microscope with phase-contrast optics	Borkman (1994), Borkman *et al.* (1993), Turner *et al.* (1995)
Screened phytoplankton	UMD	Cells/L	Olympus BH-2 compound microscope with phase-contrast optics	Bowen *et al.* (1998)
Rapid phytoplankton	UMD	Cells/L (approx.)	Olympus BH-2 compound microscope with phase-contrast optics	Bowen *et al.* (1998)
Zooplankton	UMD	$Indiv./m^3$	Wild M-5 dissecting microscope	APHA *et al.* (1989)
In Situ Conductivity	Battelle	$mmho^s/cm$	Seabird 9 CTD	Seabird CTD Manual
Temperature	Battelle	C	Seabird 9 CTD	Seabird CTD Manual
Pressure	Battelle	m	Seabird 9 CTD	Seabird CTD Manual
Dissolved oxygen	Battelle	mb/L	Seabird 13	Weiss (1970)
Chlorophyll fluorescence	Battelle	µg/L	Wetstar	Wet Lab Manual
Transmissometry	Battelle	m-1	Seatech 20-cm	Seatech Manual
In situ irradiance	Battelle	µEm-2sec-1	Biospherical QSR-200L	Biospherical Manual
Surface irradiance	Battelle	µEm-2sec-1	Biospherical QSP-240	Biospherical Manual
Altimeter	Battelle	m	Data Sonic PSA-916	Data Sonic Manual
Bottom depth	Battelle	m	Furuno FCV-52	Furuno Manual
Navigational position	Battelle		Northstar 942X	Northstar Manual
Secchi	Battelle	m	30-cm White Disk	Bowen *et al.* (1998)
Sigma-T	Battelle	null	Seabird 9 CTD	Fofonoff and Millard (1983)
Salinity	Battelle	PSU	Seabird 9 CTD	Fofonoff and Millard (1983)

Table 6. Water Column Sampling Frequency

	Station Type Code										Analyses Per Year
	Nearfield			Farfield							
	A	E	P	D	E	F	G	R	P	Z	
No. station types per survey	5	14	2	8	12	2	2	1	1	2	--
Number of surveys per year	17	17	17	6	6	6	6	6	6	3	--
Subsample Analysis	**Number of Analyses per Station**										
Dissolved Inorganic Nutrients	5	5	5	5	5	5	3	5	5	0	2541
Dissolved Organic Carbon Total Dissolved Nitrogen Total Dissolved Phosphorous Particulate Organic Carbon Particulate Organic Nitrogen Particulate Phosphorous Biogenic Silica	3	0	3	3	0	0	3	0	3	0	555
Chlorophyll *a*/phaeophytin *a*	5	0	5	5	0	0	3	0	5	0	901
Total suspended solids	3	0	3	3	0	0	3	0	3	0	555
Dissolved oxygen	5	0	5	5	0	5	3	5	5	0	991
Phytoplankton – whole water	0	0	2	2	0	0	2	0	2	0	200
Phytoplankton – screened water	0	0	2	2	0	0	2	0	2	0	200
Urea	0	0	2	2	0	0	2	0	2	0	200
Zooplankton	0	0	1	1	0	0	1	0	1	1	106
Respiration	0	0	3	0	0	0	0	3	3	0	138
Photosynthesis	0	0	5	0	0	0	0	0	5	0	200
Hydrographic profiles: Conductivity Temperature Pressure Dissolved oxygen Chlorophyll fluorescence Transmissometry *In situ* irradiance Surface irradiance Sigma-T Salinity Altimeter Bottom depth Navigational position	1	1	1	1	1	1	1	1	1	1	519
Secchi	0	0	0	1	1	1	1	1	1	1	162

8.0 PROJECT FISCAL INFORMATION

This project is being carried out under the Harbor and Outfall Monitoring contract (Contract No. S274) between MWRA and Battelle Duxbury Operations.

9.0 SCHEDULE OF ACTIVITIES AND DELIVERABLES

Table 7 lists the delivery schedule for the various water-column monitoring reports. Table 8 lists the planned schedule for all farfield and nearfield surveys and related deliverables.

Table 7. Schedule of Data Reports, Data Exports, and Synthesis Reports

Deliverable	Survey Period	Due Date
Survey-Related Reports		
Survey Plans	Each survey	2 weeks prior to survey
Survey Email Summaries	Each survey	7 days after survey
Survey Reports – Draft	Each survey	14 days after survey
Survey Reports – Final	Each survey	14 days after receipt of comments
Data Reports and Exports		
Nutrient Data Reports	February – March	May
	April – May	July
	June – July	September
	August – September	November
	October – December	February
Nutrient Data Exports	As above	1 month after Data Report
Respiration/Productivity Data Report	February – March	May
	April – May	July
	June – July	September
	August – September	November
	October – December	February
Respiration/Productivity Data Exports	As above	1 month after Data Report
Plankton Data Report	February – March	May
	April – May	July
	June – July	September
	August – September	November
	October – December	February
Plankton Data Exports	As above	1 month after Data Report
Synthesis or Interpretive Reports		
Annual Whale Observation – Draft	February - December	January
Annual Whale Observation –Final		30 days after receipt of comments
Semiannual Water Column – Draft	February – July	October
Semiannual Water Column – Final		30 days after receipt of comments
Semiannual Water Column – Draft	August - December	March
Semiannual Water Column – Final		30 days after receipt of comments
Annual Water Column – Outline	February – December	April
Annual Water Column – Draft		May
Annual Water Column – Final		30 days after receipt of comments

Table 8. Schedule of Water Column Surveys and Related Survey Reports

Survey ID	Additional Surveys Combined	Plan	Planned Due Date[a]			
			Date Start	Summary	Draft Report	Final Report
WF981	WN981	01/26/98	02/02/98	02/12/98	02/19/98	03/21/98
WF982	AV981, WN982	02/16/98	02/23/98	03/05/98	03/12/98	04/11/98
WN983	None	03/11/98	03/18/98	03/25/98	04/01/98	05/01/98
WF984	WN984	03/24/98	03/31/98	04/09/98	04/16/98	05/16/98
WN985	AV982	04/16/98	04/23/98	04/30/98	05/07/98	06/06/98
WN986	PT982	05/07/98	05/14/98	05/21/98	05/28/98	06/27/98
WF987	AV983, WN987	06/09/98	06/16/98	06/25/98	07/02/98	08/01/98
WN988	None	06/26/98	07/03/98	07/10/98	07/17/98	08/16/98
WN989	None	07/14/98	07/21/98	07/28/98	08/04/98	09/03/98
WN98A	PT983	07/31/98	08/07/98	08/14/98	08/21/98	09/20/98
WF98B	AV984, BF981, WN98B	08/10/98	08/17/98	08/27/98	09/03/98	10/03/98
WN98C	None	08/26/98	09/02/98	09/09/98	09/16/98	10/16/98
WN98D	None	09/16/98	09/23/98	09/30/98	10/07/98	11/06/98
WF98E	PC98A, AV985, WN98E	09/29/98	10/06/98	10/15/98	10/22/98	11/21/98
WN98F	None	10/21/98	10/28/98	11/04/98	11/11/98	12/11/98
WN98G	PC98B, PT984	11/18/98	11/25/98	12/02/98	12/09/98	01/08/99
WN98H	PC98C, AV986	12/09/98	12/16/98	12/23/98	12/30/98	01/29/99
WF991	WN991	01/25/99	02/01/99	02/11/99	02/18/99	03/20/99
WF992	PC992, AV991, BC991, WN992	02/15/99	02/22/99	03/04/99	03/11/99	04/10/99
WN993	None	03/10/99	03/17/99	03/24/99	03/31/99	04/30/99
WF994	PC993, WN994	03/23/99	03/30/99	04/08/99	04/15/99	05/15/99
WN995	PC994, AV992	04/15/99	04/22/99	04/29/99	05/06/99	06/05/99
WN996	PC995, PT992	05/07/99	05/14/99	05/21/99	05/28/99	06/27/99
WF997	PC996, AV993, WN997	06/08/99	06/15/99	06/24/99	07/01/99	07/31/99
WN998	None	06/25/99	07/02/99	07/09/99	07/16/99	08/15/99
WN999	PC997	07/14/99	07/21/99	07/28/99	08/04/99	09/03/99
WN99A	PT993	07/29/99	08/05/99	08/12/99	08/19/99	09/18/99
WF99B	PC998, AV994, BF991, WN99B	08/09/99	08/16/99	8/26/99	09/02/99	10/02/99
WN99C	None	08/26/99	09/02/99	9/9/99	09/16/99	10/16/99
WN99D	PC999	09/16/99	09/23/99	9/30/99	10/07/99	11/06/99
WF99E	PC99A, AV995, WN99E	09/28/99	10/05/99	10/14/99	10/21/99	11/20/99
WN99F	None	10/21/99	10/28/99	11/4/99	11/11/99	12/11/99
WN99G	PT994	11/15/99	11/22/99	11/29/99	12/06/99	01/05/00
WN99H	PC99C, AV996	12/07/99	12/14/99	12/21/99	12/28/99	01/27/00
WF001	WN001	01/24/00	01/31/00	2/10/00	02/17/00	03/18/00
WF002	PC002, AV001, BC001, WN002	02/14/00	02/21/00	3/2/00	03/09/00	04/08/00
WN003	None	03/09/00	03/16/00	3/23/00	03/30/00	04/29/00
WF004	PC003, WN004	03/21/00	03/28/00	4/6/00	04/13/00	05/13/00
WN005	PC004, AV002	04/13/00	04/20/00	4/27/00	05/04/00	06/03/00
WN006	PC005	05/04/00	05/11/00	5/18/00	05/25/00	06/24/00
WF007	PC006, AV003, WN007	06/06/00	06/13/00	6/22/00	06/29/00	07/29/00
WN008	None	06/23/00	06/30/00	7/7/00	07/14/00	08/13/00
WN009	PC007	07/12/00	07/19/00	7/26/00	08/02/00	09/01/00
WN00A	None	07/27/00	08/03/00	8/10/00	08/17/00	09/16/00
WF00B	PC008, AV004, BF001, WN00B	08/07/00	08/14/00	8/24/00	08/31/00	09/30/00
WN00C	None	08/25/00	09/01/00	9/8/00	09/15/00	10/15/00
WN00D	PC009	09/14/00	09/21/00	9/28/00	10/05/00	11/04/00
WF00E	PC00A, AV005, WN00E	09/26/00	10/03/00	10/12/00	10/19/00	11/18/00
WN00F	None	10/19/00	10/26/00	11/2/00	11/09/00	12/09/00
WN00G	PC00B	11/13/00	11/20/00	11/27/00	12/04/00	01/03/01
WN00H	PC00C, AV006	12/05/00	12/12/00	12/19/00	12/26/00	01/25/01

WN: water column nearfield; WF: water column farfield; AV: anthropogenic virus; PT: fecal coliform transect; BF; farfield benthic; PC: fecal coliform conditional; BC: Nearfield contaminant special.
[a] Tentative dates. Actual dates will be determined based on the survey completion date.

10.0 PROJECT ORGANIZATION AND RESPONSIBILITIES

The Water Column Monitoring tasks will be accomplished through the coordinated efforts of several organizations (Figure 5). Dr. Mike Mickelson is the MWRA Project Manager and the MWRA Water Column Project Area Manager. He will be informed of all matters pertaining to work described in this CW/QAPP. Mr. Ken Keay is the MWRA Deputy Project Manager and will serve as a backup to Dr. Mickelson. Ms. Wendy Leo is the MWRA EM&MS Database Manager.

Dr. Carlton Hunt is the Battelle Project Manager and is responsible for the overall performance of this project. The Battelle Quality Assurance Officer for the project is Ms. Rosanna Buhl. For this task, Ms. Buhl is responsible for reviewing data reports and QA Statements submitted by members of the Water column monitoring team for completeness and adherence to the CW/QAPP. She is also responsible for reviewing the data reports for accuracy and completeness. Mr. Wayne Trulli is the Battelle Field Manager responsible for all Battelle field collections. Ms. Heather Trulli, Battelle's Laboratory Manager, is responsible for overseeing all laboratory activities in the contract. Ms. Ellie Baptiste-Carpenter is Battelle's Database Manager. The key contacts at each of the supporting laboratories are shown in Figure 5. Addresses, telephone (and fax) numbers, and Internet addresses, as well as specific project roles and responsibilities, are defined in detail in the HOM3 Program Management Plan (Battelle 1998).

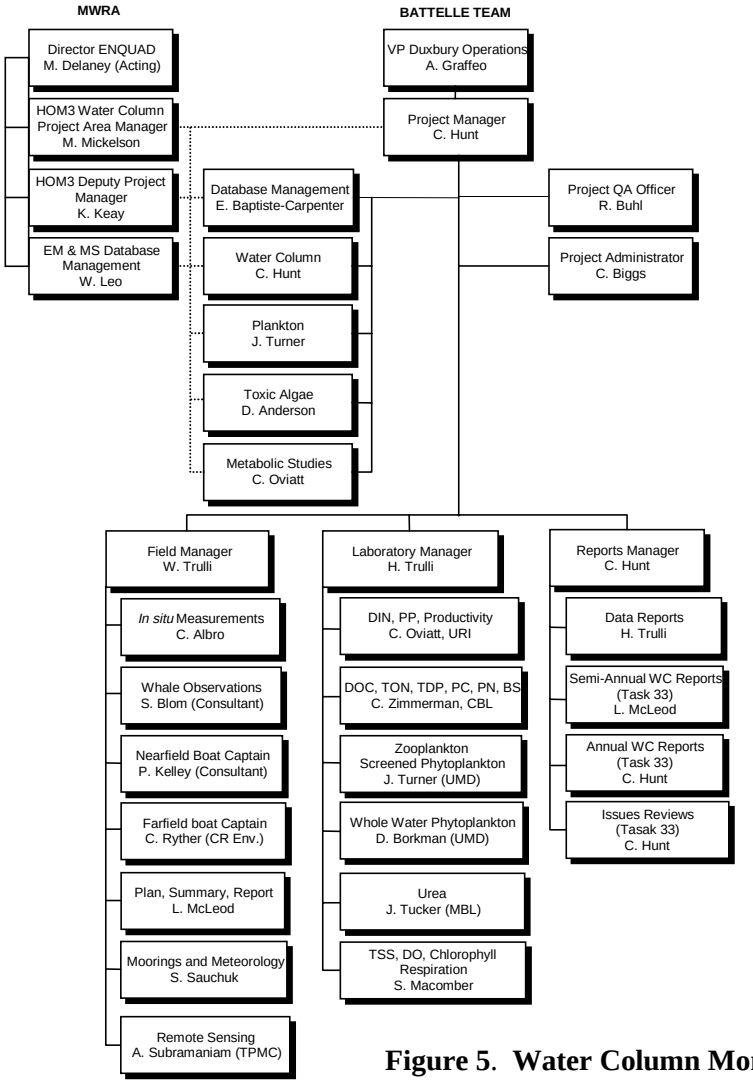


Figure 5. Water Column Monitoring Organization

11.0 DATA QUALITY REQUIREMENTS AND ASSESSMENTS

To ensure that all data generated during the conduct of surveys, analyses, and reporting are of the highest quality, data will be examined in terms of precision, accuracy, completeness, comparability, and representativeness. These terms are defined in the HOM3 Quality Management Plan (Battelle 1998). The application of these data quality measures is described below.

11.1 Navigational and Hydrographic Data

11.1.1 Precision and Accuracy

Based on manufacturer specifications or Battelle's experience, precision and accuracy objectives for navigation and hydrographic samplings are presented in Table 9. Section 12 provides details on relevant sampling procedures to ensure data quality and Section 14 contains instrument calibration methods and specifications.

Table 9. Accuracy and Precision of Instrument Sensors and Secchi Disc

Sensor	Reporting Units	Range	Accuracy	Precision
Pressure (depth)	decibars	0 to 1000	0.60	0.1
Temperature	ºC	-2 to +30	0.015	0.01
Conductivity	mS/cm	0.5 to 65	0.02	0.01
Transmissometer (20-cm)	m^{-1}	0 to 40	0.20	0.01
Dissolved oxygen	mg/L	0 to 15	0.50	0.05
In-situ irradiance	$\mu E\ m^{-2}\ s^{-1}$	0 to 4000	10	1
On-deck irradiance	$\mu E\ m^{-2}\ s^{-1}$	0 to 4000	10	1
Fluorometer	$\mu g/L$	0.1 to 100	50% of reading*	0.01
Echosounder (depth)	m	0 to 200	2	0.1
DGPS Navigation	degree	coastal	1.8×10^{-5} degrees	1.8×10^{-5}
Altimeter	m	0 to 100	1	0.1
Secchi disk (30-cm, white)	m	0 to 40	0.5	0.5

*When compared to wet chemistry results.

11.1.2 Completeness

Battelle's navigation software system outputs navigation positions at an interval of 2–s. The software system will display all position fixes and save these fixes in an electronic file during hydrocasts and sampling operations. The project's time interval requirement for obtaining positions during sampling is 1–min. Thus, even with a few bad data streams from the DGPS navigation system to the computer, the software will provide enough fixes within each 1-min period for 100% data collection. During transit between stations, the software system will save vessel coordinates in an electronic file every five minutes.

Because hydrographic data are acquired electronically and monitored in real time, no loss of data is expected. With the sampling rates of the CTD (4 Hz) and navigation systems (2 s intervals), sufficient data will be acquired to locate the depth of the pycnocline. Stations will not be occupied if CTD measurements (at a minimum) cannot be obtained. If instrument malfunctions occur and operations are modified or suspended during any survey day, a decision on modification of activities for that survey will be made with consultation and agreement of MWRA, whenever possible. A 10% loss of hydrographic and navigation data over the entire program is not expected to compromise the objectives of the program.

11.1.3 Comparability

Latitude/longitude positions will be recorded. These positions will be comparable to positions obtained by previous MWRA monitoring activities as well as by other researchers that have used or are using differentiated GPS at these stations. The station locations listed in Tables 2 and 3 are targets and sampling will be attempted within 300 m of the targets as visualized on the BOSS navigation display. Objectives will not be compromised if conditions force sampling within 600 m from the targets.

The electronic measurement instruments that will be used during the water quality monitoring surveys are similar to the instruments that have been used by MWRA contractors from 1992 through 1997 (Albro *et al.* 1993 and Bowen *et al.* 1998). Except for dissolved oxygen and chlorophyll fluorescence sensor values, the instrumentation data reduction methods are based on laboratory or vendor calibrations. To improve the representativeness of the electronic dissolved oxygen and chlorophyll fluorescence values to wet chemistry data collected during each survey, the electronic data is post-calibrated using the wet chemistry data. To maintain comparability with the 1995 through 1997 data, the same post-calibration methods will be used (Bowen *et al.* 1998). Thus, the data should be consistent with and comparable to previous studies. During review and synthesis of the survey data, the results will be compared with the general ranges of water property data obtained from previous MWRA studies.

MWRA recently sponsored a comparability study of three different Secchi disks. The study findings are in Appendix B.

11.1.4 Representativeness

The representativeness of the sampling program design is detailed in the Outfall Monitoring Plan (MWRA 1997b). Representativeness will also be ensured by proper handling, storage, and analysis of samples so that the materials analyzed reflect the collected material.

11.2 Water Sampling

11.2.1 Precision and Accuracy

Precision and accuracy of water sampling procedures are not directly quantified, but are ensured by the collection procedures. The sampling objective is to obtain uncontaminated samples representative of their location. Procedures will follow standard methods that can achieve this objective. Each sample will be clearly labeled with a unique sampling identifier (survey ID and sample number) that will allow the sample to be traced from collection through analysis to reporting. All samples will be handled and stored according to the appropriate protocols.

11.2.2 Completeness

The nearfield surveys will be considered complete if all stations except seven E-type stations are sampled. All farfield stations must be sampled for the survey to be considered complete.

At each station, discrete samples will be collected at 5 depths (only 3 depths collected at Stations F30 and F31) based on positions relative to a subsurface chlorophyll maximum usually associated with the presence of a pycnocline separating surface and bottom water layers. In the event of sample loss or equipment malfunction, the Chief Scientist will determine the need for appropriate corrective action (*e.g.*, resampling) and will record such action in the survey notebook. If no distinct vertical hydrographic structure is apparent from the real-time *in situ* sampling, the hydrocast will not be resampled at the discretion of the Chief Scientist. In all cases, the objectives of the project will not be compromised if

representative surface and mid-depth ("chlorophyll maximum" if present) samples for nutrient and biological studies, and measurements of bottom-water DO are successfully collected.

11.2.3 Comparability

Collection of samples for chlorophyll and DO measurements coincidentally with *in situ* electronically captured data will allow field calibration of the electronic sensors. Nutrient concentrations (dissolved and particulate) will be comparable to data from other recent surveys of the study area because standardized sampling procedures will be employed. Reporting units concentrations will follow standard convention for most oceanographic studies.

Comparability of the sampling procedures with previous studies will be achieved through adherence to procedures that are based on documented standard methods (*e.g.*, EPA or ASTM methods) or on methods previously described in the scientific literature or HOM monitoring program documents. Comparability throughout the project will be achieved through adherence to this CW/QAPP.

11.2.4 Representativeness

Water samples will be collected, handled, and transported using procedures that will ensure that resulting data represent the sample material collected.

11.3 Laboratory Program

Table 10 summarizes the laboratory data quality objectives for water column monitoring. Section 12 provides additional details on the analytical procedures (*e.g.*, prepared standards) that will ensure data quality, and Section 14 describes instrument calibration methods.

11.3.1 Precision and Accuracy

11.3.1.1 Particulate Nutrients

There is no SRM for particulate nutrients, but marine sediment SRM is tested for C, N, and P prior to start of project analysis.

11.3.1.2 Primary Productivity

URI will provide DQO information on the primary productivity method once the revised procedure agreed to in mid-January 1998 is fully tested using the photosynthetrons.

11.3.1.3 Whole-Water Phytoplankton

Based on a study conducted by Guillard (1973), counts of 400 phytoplankton cells will provide a precision of $\pm$10% of the mean. For this program, a minimum of 400 entities (single cells, chains, or colonies), will be tallied for each sample. Unicellular forms (*e.g.*, *Cryptomonas*, microflagellates), aggregate forms (*e.g.*, *Phaeocystis*), and chained forms (*e.g.*, *Skeletonema*) will each count as one entity towards the 400-entities-counted-per-sample minimum tally. To increase precision of the abundance estimates for the most abundant taxa, when practical at least 75 entities of each of the three most abundant taxa will be counted in each sample.

Table 10. Data Quality Objectives

Quality Control Sample Type		Frequency	Data Quality Indicator	Corrective Action
Procedural Blanks				
	Dissolved nutrients	1 per batch of 20	≤5 times MDL	Results examined by subcontractor lab manager, task leader, or project manager. Corrective action (*e.g.*, re-extraction, reanalysis, data qualifier) is documented.
	Urea	10%	≤0.1 μM	
	Total suspended solids (DI water and seawater)	3/day	≤5 times MDL	
Filter Blanks				
	Particulate nutrients	1 per batch of 20	≤5 times MDL	As above
	Biogenic silica	1 per batch of 20	≤5 times MDL	
	Chlorophyll *a*/phaeophytin	Once daily	≤5 times MDL	
	Total suspended solids	1 per batch of 20	≤5 times MDL	
Prepared Standards and SRM				
	Dissolved nutrients	Twice per year	85% - 115% recovery	As above
	Urea	Analysis initiation	≤10% RPD	
	Chlorophyll *a*/phaeophytin	1 per batch of 20	≤15% RPD	
Laboratory Duplicates				
	Particulate nutrients	10% of samples	≤15% RPD	As above
	Urea	1 per batch of 20	≤15%RPD	
	Chlorophyll *a*/phaeophytin	1 per batch of 20	≤15% RPD	
	Total suspended solids	Every sample	≤10% RPD	
Laboratory Triplicates				
	Dissolved nutrients	All samples	≤2% RPD	As above
	Dissolved oxygen	5% of samples and begin/end of each survey	≤5% CV	
Field Duplicates				
	Particulate carbon Particulate nitrogen Particulate phosphorus Biogenic silica Chlorophyll a/phaeophytin	Each mid-depth	<50% RPD	Data qualified with "r"

NA: Not Applicable
Percent Recovery =[(amount recovered - amount in background matrix)/amount spiked] × 100%.
Relative Percent Difference (RPD) = [(absolute value (replicate 1 - replicate 2) × 2/(replicate 1 + replicate 2)] × 100%.
Coefficient of Variation (CV) = (standard deviation of the sample concentration / mean sample concentration) × 100%.

11.3.2 Completeness

It is expected that 100% of the samples collected and intended for analysis will be analyzed. However, a sample loss of <10% for the entire project will not compromise the objectives of the project.

11.3.3 Comparability

Data will be directly comparable to results obtained previously at the same or similar sites in Massachusetts Bay and to those of similar studies conducted in Cape Cod Bay (Albro *et al.* 1993; Bowen *et al.* 1998), because field program design and analytical procedures are similar or identical. In addition,

the use of written standardized procedures ensures that sample preparation and analyses will be comparable throughout the project and with other projects. Specific, potential comparability issues are addressed below.

11.3.3.1 Nutrients

At the request of MWRA, CBL performed a study to determine potential errors introduced through the use of borosilicate vessels versus Teflon™ vessels for the total dissolved nitrogen and phosphorus digestions. CBL found that no contamination resulted from the use of borosilicate digestion vessels. A summary of the study is in Appendix C.

11.3.3.2 Total Suspended Solids

Battelle conducted a comparison study of different filters to determine which filters produced the most reliable results. The study found that Nuclepore filters were the preferred filters for TSS analyses. A summary of the study is in Appendix D.

11.3.3.3 Productivity

URI conducted a study of the reliability of using reduced sample volumes to measure primary productivity using ^{14}C. The study found that analyses using 5-mL samples could produce results that were comparable to analyses using larger sample volumes. A summary of the study is in Appendix E.

URI measured the effects of sample holding time and increased incubation time on measurements of primary productivity using the photosynthetrons at URI. The results, summarized below, show that sample analysis must begin within 6 h of sample collection and incubation between 0.5 h and 2 h produce comparable results.

Incubation Time	
Time (h)	**Productivity ($gC/m^2/h$)**
0.5	0.195
1	0.207
1.5	0.182
2	0.212

Holding Time	
Time (h)	**Productivity ($gC/m^2/h$)**
0	0.207
4	0.182
6	0.210
8	0.177

11.3.3.4 Whole Water Phytoplankton

An inter-laboratory comparison study was conducted to determine the comparability between the whole-water-phytoplankton analytical method described in this CW/QAPP and the method described by Bowen *et al.* (1998). The study results demonstrated that the two methods produced comparable results. (Dr. Donald Anderson, WHOI, Personal Communication; April 1998, Appendix F). Additional comparisons will be performed on approximately six samples collected between March and May 1998; these analyses will document comparability of analyses on fresh (*i.e.*, recently collected) samples versus stored (collected weeks to months prior to comparison analysis) samples.

In tandem with the additional comparisons mentioned above, a time series of analyses will be performed to address the condition of microflagellates during sample storage. Microflagellates will be enumerated at approximately weekly intervals over the course of two to three months. The results of the two additional comparison studies will be incorporated into Revision 1 of this CW/QAPP.

11.3.4 Representativeness

Representativeness is addressed primarily in sampling design. The laboratory measurements that will be made during the water quality monitoring task have already been used in many systems to characterize eutrophication effects on the water column and are, therefore, considered to yield data representative of the study area. Representativeness will be ensured also by proper handling, storage (including appropriate preservation and holding times), and analysis of samples so that the material analyzed reflects the material collected as accurately as possible.

Deviations from the analytical scheme described in this CW/QAPP will be noted in the laboratory records associated with analytical batches and in the QA statements and will be discussed in the quarterly QA/QC Corrective Action reports.

11.3.5 Sensitivity

Sensitivity is the capability of methodology or instrumentation to discriminate among measurement responses for quantitative differences of a parameter of interest. The method detection limits (MDL) (Table 11) provide the sensitivity goals for the proposed procedures.

Table 11. Method Detection Limits

Analysis	MDL
Dissolved ammonia	0.02 µM
Dissolved inorganic nitrate	0.01 µM
Dissolved inorganic nitrite	0.01 µM
Dissolved inorganic phosphorus	0.01 µM
Dissolved inorganic silicate	0.02 µM
Dissolved organic carbon	20 µM
Total dissolved nitrogen	1.43 µM
Total dissolved phosphorus	0.04 µM
Particulate carbon	5.27 µM
Particulate nitrogen	0.75 µM
Particulate phosphorus	0.04 µM
Biogenic silica	0.32 µM
Urea	0.2 µM
Chlorophyll *a* and phaeophytin (EDL)	0.036 µg/L
Total suspended solids	0.1 mg/L

EDL: estimated detection limit

12.0 SAMPLING AND ANALYTICAL PROCEDURES

Methods for collection and analysis of samples are described in the following sections. Analyses will be performed by Battelle, CBL, MBL, URI and UMD as defined below.

12.1 Field Sampling and Measurements

12.1.1 Navigation

Vessel positioning during sampling operations will be accomplished with the BOSS navigation system. This system consists of a Northstar DGPS interfaced to the BOSS computer. The GPS receiver has six dedicated channels and is capable of locking onto six different satellites at one time. To correct the GPS calculations, the Northstar DGPS will receive correction data from one of three USCG DGPS broadcast sites: Montauk Point, NY, Chatham, MA, or Portsmouth Harbor, NH (Figure 6). This capability ensures strong signal reception, and accurate and reliable positioning with 2-s updates.

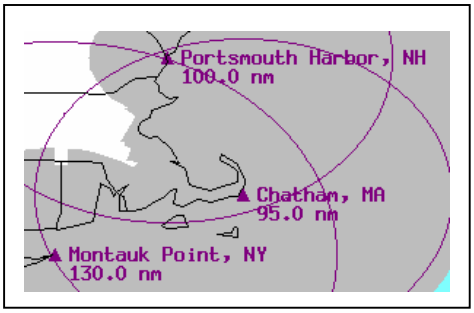


Figure 6. DGPS Master Stations Coverage

12.1.2 Vessel Handling

Boston Harbor, Massachusetts Bay, and Cape Cod Bays are heavily trafficked by commercial, fishing, and recreational vessels. Endangered whales, as well as numerous other marine mammals seasonally frequent the Bays. The licensed boat captain will operate the vessel in a professional manner at all times during surveys to minimize the possibility of collisions with other traffic or with marine mammals. Also required by National Marine Fisheries Service's rules, the vessel will maintain a minimum distance of 500 yards from right whales. If a right whale is within 500 yards of a sampling station, the vessel will wait at least 30 minutes for the right whale to move out of range or the station will be sampled as close to nominal as possible to nominal while maintaining the minimum required distance from right whales.

At each sampling station, the vessel will be positioned about 300 meters upwind/upcurrent of the target station position. The vessel heading will be selected such that the underwater unit will be deployed on the side of the boat facing the sun and relative to the prevailing seas, which will minimize cable loading. The vessel will maintain this position during the cast. If a vessel safety issue causes shading of the CTD, the shading incident will be noted in the station log. During post-processing of the hydrographic data, any shading incidents will be noted in the PROFILE.VAL_QUAL field of the EM&MS database.

12.1.3 Hydrographic Profiles

The hydrographic profile sampling equipment and data acquisition equipment consists of the following apparatus and instruments.

- Battelle-designed and –fabricated winch with 150 m of 9-conductor double-armored stainless-steel cable and sheave
- Sea-Bird 32 Carousel Water Sampling System or General Oceanics model 1015 Rosette system
- 5- and 9-L Rosette sampling bottles (*e.g.*, Go-Flo or Niskin)
- Sea-Bird CTD interface deck unit
- Sea-Bird SBE-9 CTD system (backup is Ocean Sensors OS200-CTD) equipped with the following:
  - Sea-Bird SBE-13 DO sensor, which is a Beckman polarographic type that produces an oxygen-dependent electrical current and incorporates a thermistor for determining membrane temperature

  - Sea-Bird SBE-4-01/0 conductivity cell
  - Paroscientific Digiquartz integral to the SBE-9 CTD to measure pressure
  - Sea Tech 20-cm-pathlength transmissometer that provides *in situ* measurements of optical beam transmission (related to the concentration of suspended matter in the water at the point of measurement)
  - Wet Labs WetStar chlorophyll fluorometer
  - Biospherical QSP-200L spherical quantum scalar irradiance sensor that measures underwater photosynthetically active radiation (PAR)
- Biospherical QSR 240 reference hemispherical quantum scalar irradiance sensor that measures on-deck radiation conditions (*e.g.*, due to atmospheric conditions)
- Data Sonic altimeter provides a measurement of underwater unit height from the bottom
- JRC JFV-120 dual-frequency color video echosounder to provide bathymetric measurements during vertical and horizontal profiling operations
- Computer with custom data-acquisition software (NAVSAM)
- Color printer

Battelle's software, NAVSAM© acquires data from all profile electronic-sampling-systems and navigation systems at the rate of four times per second. Once per second the software displays all of the information on a color monitor. The screen is split to show sensor data on the left and navigation data on the right (Figure 7). Once the data are acquired, they are automatically written to a data file and logged concurrently with position data from the navigation system. The navigation portion of the display will show the position of the vessel compared to the coastlines digitized from standard NOAA charts, navigation aids, preset sampling locations, and vessel track. A second monitor will be furnished to the helmsman as a steering display. During hydrocast operations, position fixes will be electronically recorded at 2-sec intervals. Hard-copy printouts of position fixes will be made during discrete sampling events such as triggering of Rosette sampling bottles. During transit between stations, position fixes and deck irradiance will be electronically recorded at 5-min intervals. Irradiance measurements will be conducted from one-half hour after sunrise to one-half hour before sunset.

12.1.4 Water Sampling

Water samples for dissolved inorganic nutrients, dissolved organic nutrients, particulate nutrients, chlorophyll *a*, TSS, DO, primary production and phytoplankton will be obtained by using an underwater unit equipped with Rosette sampling bottles. The rosette system is combined with the hydrographic profiling system. The following water sampling/hydrographic profiling procedures will be followed:

1. Before the start of each cast, each of the Rosette sampling bottles will be opened and attached to the Rosette triggering system.

2. After the vessel is positioned as described in section 12.1.2, NAVSAM will be set to the hydrographic profiling mode and a data cast file will be opened. NAVSAM will acquire data from the equipment while the underwater unit is on-deck prior to deployment. The operator will review the sensor data to verify that all sensors have reasonable readings (*i.e.*, reasonable surface irradiance, beam attenuation less than 0.5/m). These on-deck readings will be used to adjust the depth offset and match the irradiance sensors.

3. After a successful on-deck check out, the underwater unit will be lowered into the water until completely submerged and held in this position.

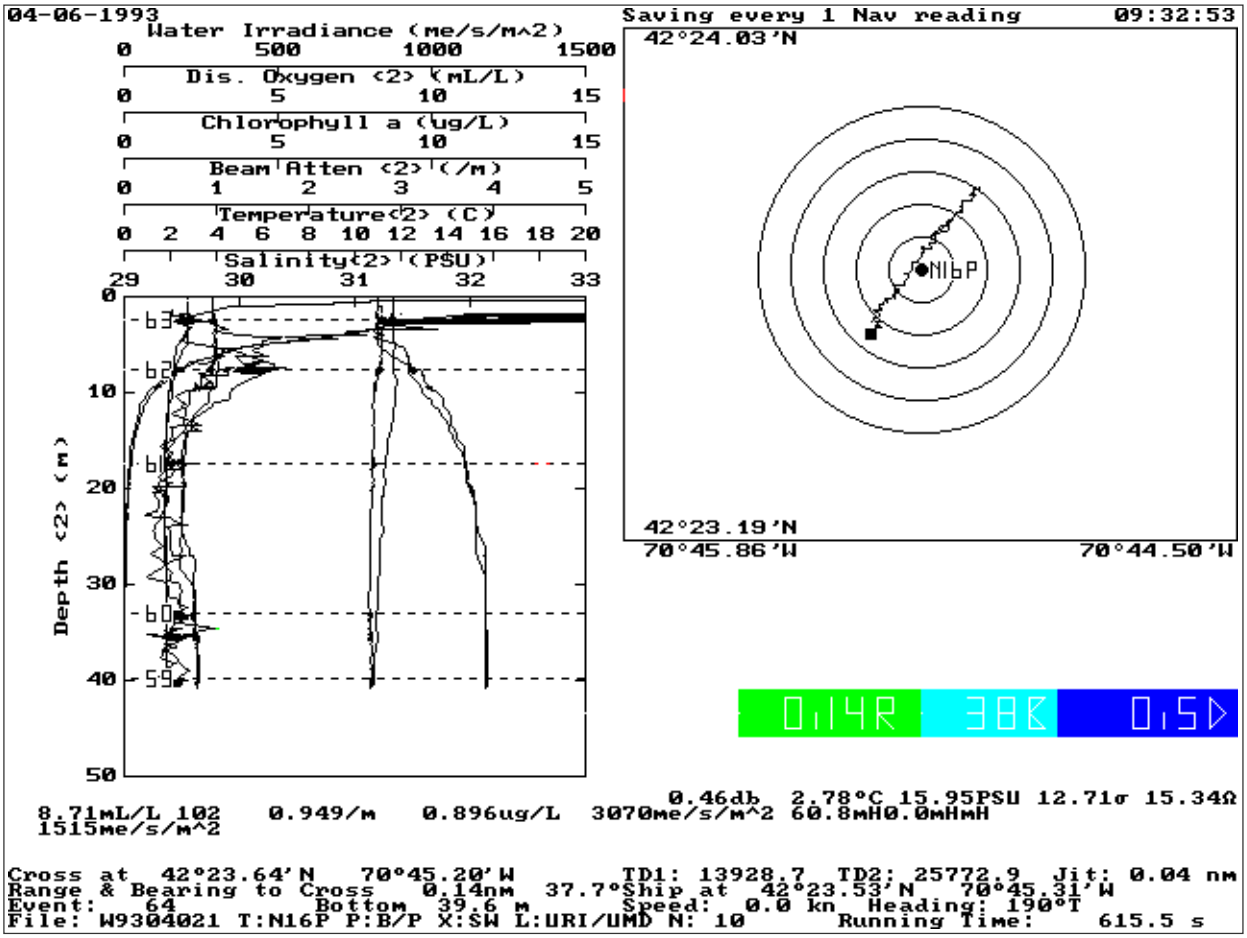
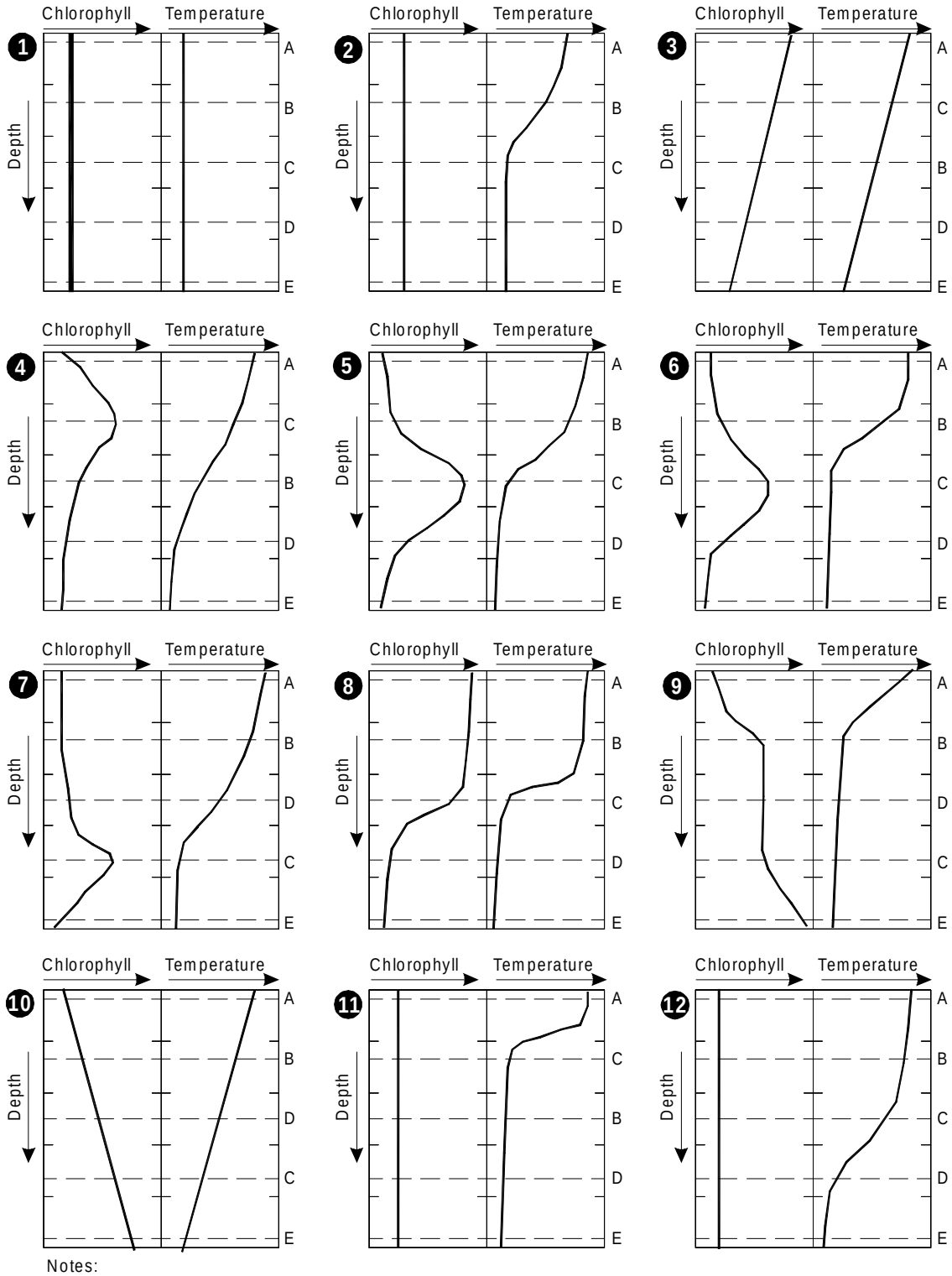


Figure 7. Sample NAVSAM Data Acquisition Screen

4. After the underwater unit has been submerged for 1-2 minutes to allow all of the sensors to equilibrate, the unit will be lowered at a descent rate of about 0.5 m/s to within 3-5 m of the bottom.

5. During the lowering, NAVSAM will record the hydrographic data and display these data on a computer screen. The chief scientist will then review the real–time display of data to determine the five water sampling depths that are based on positions relative to a subsurface chlorophyll maximum detected by *in situ* fluorometer. The 5 sampling depths are designated surface (A), mid-surface (B), mid–depth (C), mid–bottom (D), bottom (E) as listed in Table 4, although actual sampling depths would not necessarily be evenly spaced. Depending shown the profile of chlorophyll and temperature, the mid-surface and mid-depth or mid-bottom and mid-depth levels can be exchanged. Figure 8 shows plot examples and sampling depths. Scenario 4 shows an intense and shallow chlorophyll maximum. In this case, the sampling protocol for the mid-depth and mid-surface would be exchanged so that the chlorophyll maximum would receive the full suite of analyses usually allocated to the water column mid-depth. Scenario 7 shows an intense and deep chlorophyll maximum, thus the protocols for mid–depth and mid–bottom would be exchanged.



Notes:

- Mid-depth 'C' must be less than 30 meters and cannot be the bottom depth.
- At station type 'P', try to position mid-bottom 'D' at 10% incident light, mid-depth 'C' at 25% incident light, and mid-surface 'B' at 50% incident light

Figure 8. Twelve Scenarios for Selecting Sample Depths

6. During the upcast, the unit will be maintained at each of the selected five depths until the sensor readings stabilize. Typically this is 30–60 seconds. Water will be collected by closing one or more Rosette sampling bottles, depending on the water volume needed for analysis. When the Rosette deck unit indicates that the bottles are closed, this event will be flagged electronically in the NAVSAM data file. This marks the vessel position and the concurrent *in situ* water column parameters (salinity, temperature, turbidity, DO, chlorophyll *a*, irradiance, and depth) and links them to water collected in a particular set of Rosette sampling bottles. The NAVSAM software will also generate unique bar-coded sample-bottle labels for attachment to sample bottles and survey logs. Onboard processing is described in Section 12.2.
7. After collecting the surface water sample, the operator will close the data cast file.
8. The underwater unit will be recovered.
9. The NAVSAM will be put into navigation mode with a file created for transit to the next station.

12.1.5 Zooplankton Sampling

At "D", "G", "P", and "Z" type stations, a vertical–oblique zooplankton tow will be conducted with a 0.5-m diameter 102 µm-mesh net equipped with a flow meter. Tows will be in a vertical-oblique fashion, with just enough headway to keep the net stretched out. Tows will be made through approximately the upper 30 m (or less, at shallow stations) of the water column. Because nets are equipped with flow meters, net clogging is apparent when the flow meter is no longer visibly turning as the retrieved net nears the surface. In the event of net clogging due to large numbers of phytoplankton, the net will be emptied and rinsed with filtered seawater, and the a second tow will be conducted over a shorter period of time. When the net does not clog and a sample is collected successfully, the material retained by the net will be transferred to a jar as described in Section 12.2.15. The total flow through the net, the tow time, and the depth of the tow will be recorded on the zooplankton chain-of-custody form.

12.1.6 Secchi Disk

At each Farfield station, Secchi depth will be measured. A 30-cm (approximately 12 inches) diameter white disk will be lowered overboard on a line marked in 1–meter intervals. The disk will be slowly lowered over the side facing the sun. The depth at which the disk disappears, and the depth at which it reappears (after being lowered further and then raised) will be observed. The average of the two depths (disappearing and reappearing) will be recorded on the station log.

12.1.7 Whale Observation

During each nearfield survey and the first three farfield surveys of each year, a trained whale observer will conduct sighting watches while on station and during transit between stations. The sighting operations will occur during daylight hours and when the vessel is in Massachusetts Bay or Cape Cod Bay. The observer will scan the ocean surface by eye for a minimum of 40 minutes every hour. All sightings will be recorded on standardized marine mammal field sighting logs (Figure 9). Header fields for sighting logs include observer name, date and time, weather, wind speed, sea state, vessel name, heading and speed. In addition, the observer will record the vessel position every 20 minutes, time of sighting, observer position on vessel, sighting event code (on or off watch, transiting or on station), relative bearing to mammal, species name, and number of animals sighted on the sightings logs.

Marine Mammal Sightings Log

Task: Type: Date: Page ___ of ___ Observer:

Date	Time	Position at Sighting		Vessel Heading		Mammal Sighting				Weather Conditions							Glare	
mmddyy	24-h clock	Latitude (°N)	Longitude (°W)	Direction	Speed	Species	Angle Rel. to Boat	Distance (m)	No. in Group	Sea State	Wind Speed	Swell	Visibility	Cloud Cover	Rain	Fog	Angle from Boat Head.	Glare Code

Code List

Species	
Mn	Humpback whale
Bp	Finback whale
Eg	Right whale
Ba	Minke whale
Lag	Atlantic whitesided dolphin
Pp	Harbor porpoise
Gn	Pilot whale
Bn	Blue whale
Bp	Sei whale
Lal	Whitebeaked dolphin
Pv	Harbor seal
G	Gray seal
H	Hooded seal
Ha	Harp seal
UB	Unidentified baleen whale
UO	Unidentified Odontoceti
UP	Unidentified Phocid

Sea State			
0	Glass	3	1.5 - 3 ft
1	Catpaw	4	3 - 6 ft
2	3 in - 1.5 ft	5	> 6 ft.

Wind Speed (knots)			
0	0 – 5	3	15 – 20
1	5 – 10	4	20 - 25
2	10 – 15	5	> 25

Swell (feet)			
0	None	2	3 – 6
1	1 – 3	3	> 6

Glare			
0	None	2	Moderate
1	Mild	3	Severe

Visibility (miles)			
0	None	4	3 – 5
1	< ¼	5	5 – 10
2	¼ - 1	6	10
3	1 - 3	7	Unlimited

Figure 9. Example of Marine Mammal Sightings Log and Relevant Codes

The sampling vessels will operate according to protocols mandated by the Commonwealth of Massachusetts regarding right whales (Appendix G).

12.2 Onboard Sample Processing

Depending on the subsampling requirements at each station, some or the entire following onboard sample processing procedures will be conducted. Appendix A Tables A1 and A2 lay out the required subsampling required for nearfield and farfield water column surveys, respectively.

For onboard processing of nutrients, chlorophyll, urea, and total suspended solids, water from the Rosette sampling bottles are transferred to one to four 1–L opaque polyethylene jars. These transfer jars will be rinsed three times with Rosette sampling bottle water before filling with water up to the neck of the jar. The filtration apparatus will be rinsed between sampling stations by using deionized water. The filtrate sample bottles will be rinsed three times with filtrate prior to filling.

Figure 10 summarizes the onboard processing of the dissolved and particulate nutrient subsamples from the four 1-L opaque polyethylene jars. The figure summarizes Battelle SOP No. 5-266, *Nutrient Sample Processing*. Sample volumes, containers, and storage conditions are listed in Table 12.

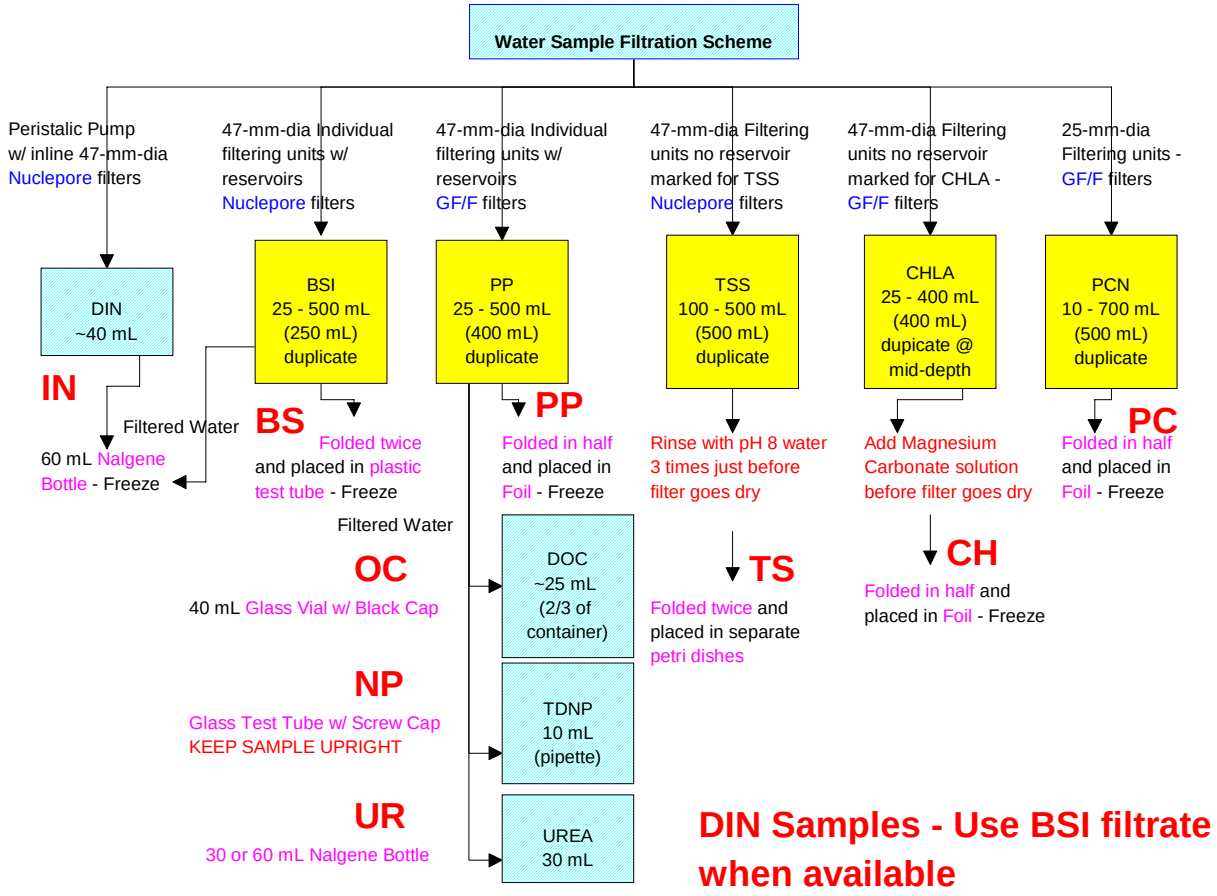


Figure 10. Onboard Processing Flow Chart

Table 12. Sample Volumes, Containers, and Processing for Field Samples

Parameter	Station Types	Sample Volume (Target) (mL)[a]	Sample Containers[c]	Shipboard Processing/ Preservation[c]	Maximum Holding Time to Analysis
Hydrographic Profiles[b]	All	NA	NA	Record data to floppy diskette.	NA
Secchi	Farfield	NA	NA	Record in field log.	NA
Subsamples from PVC Rosette Bottles					
Dissolved inorganic nutrients	All but Z	40	60-mL polyethylene bottle	Pass through a Nuclepore membrane filter. Freeze until analysis.	28 days
Dissolved organic carbon	A, D, G, P	30 (25)	40-mL glass vial	Pass sample through a GF/F. Freeze filtrate until analysis.	28 days
Total dissolved phosphorus and nitrogen	A, D, G, P	10	20 to 50-mL glass digestion tube	Pass sample through a GF/F. Freeze filtrate until analysis.	28 days
Particulate organic carbon and nitrogen	A, D, G, P	10 – 700 (500)	Whatman GF/F in foil	Pass through a GF/F. Freeze filter until analysis.	28 days
Particulate phosphorus	A, D, G, P	25 – 500 (400)	Whatman GF/F in foil	Pass sample through a GF/F. Freeze filter until analysis.	28 days
Biogenic silica	A, D, G, P	25 – 500 (250)	Nuclepore filter in 50-mL polyethylene centrifuge tube	Pass sample through Nuclepore filter. Freeze filter until analysis.	90 days
Chlorophyll *a* and phaeopigments	A, D, G, P	25 – 400 (400)	Whatman GF/F in foil	Pass through GF/F filter. Fix with a saturated $MgCO_3$ solution. Freeze filter until analysis	4 weeks
Urea	D, G, P	40 (30)	60-mL polyethylene bottle	Pass sample through glass fiber filter. Freeze filtrate until analysis.	30 days
Total suspended solids	A, D, G, P	100 – 500 (500)	Nuclepore filter in a Petri dish	Pass sample through a Nuclepore filter. Store in petri dish at room temperature.	6 months
Dissolved oxygen	A, D, F, G, P, R	300	300 mL glass BOD bottle	Fix per Oudot *et al* (1988). Titrate 2-24h later.	24 hours
Respiration	R, P	300	300 mL glass BOD	Incubate in dark at in-situ temperature for 3-7 days. Fix initial samples on board and titrate within 24 h; fix and titrate as per initial samples.	24 hours
Primary Production by ^{14}C	P	5	1-L glass bottle	Store water in 1-L dark bottle; keep cool up to and during transport to URI for incubation.	< 6 hours
Phytoplankton (whole water)	D, G, P	850	1000 mL HDPE bottle	Preserve with Utermöhl's solution.	6 months
Phytoplankton (screened water)	D, G, P	4000	1000 mL HDPE bottle	Strain through a 20-µm mesh netting; wash retained organisms into bottle. Preserve with Utermöhl's solution.	6 months
Rapid phytoplankton	Station N18 mid-depth	4000	1000-mL HDPE bottle	Strain through a 20-µm mesh netting; wash organisms into bottle; preserve with Utermöhl's solution.	6 days
Sample from vertical net tow					
Zooplankton	D, G, Z, P	800	1000-mL HDPE bottle	Wash with screened seawater into jar. Fix with formalin.	6 months

HDPE: High-density polyethylene
GF/F: pre-ashed glass fiber filter
[a]Volume processed for analysis. Total volumes removed from Rosette sampling bottles are listed in Appendix A Tables A1-A2.
[b] Conductivity, temperature, pressure, dissolved oxygen, chlorophyll a fluorescence, transmissometry, *in situ* irradiance, surface irradiance, bottom depth, navigational position
[c]Name brand items (*e.g.*, Nuclepore, Whatman) may be substituted with comprable items from a different manufacturer.

12.2.1 Dissolved Inorganic Nutrients

A 60-mL syringe will be used to inject sample water from a transfer jar, through an in-line filter (Nuclepore 47–mm–diameter, 0.4-µm-membrane-fiber filter) and into a 60-mL white polyethylene bottle. After rinsing the bottle three times, 40 mL of the remaining sample will be filtered into the bottle for analysis. Alternatively, according to Battelle SOP No. 5-266, the filtrate from the biogenic silica preparation may be used for these samples. The sample bottle will be labeled and the sample will be frozen. The samples will remain frozen until analyzed.

12.2.2 Dissolved Organic Carbon

Samples for dissolved organic carbon (DOC) will be processed according to Battelle SOP No. 5-266, *Nutrient Sample Processing*. A 25-mL aliquot will be obtained from the particulate phosphorous filtrate. The sample will be passed through a Whatman 47–mm–diameter GF/F and collected in a polysulfon filtration flask. A clean 40-mL glass vial will be rinsed three times with filtrate then filled with approximately 30 mL of filtrate. Samples will be frozen onboard and stored frozen until analysis.

12.2.3 Total Dissolved Nitrogen and Phosphorus

Samples for total dissolved nitrogen and phosphorus will be processed according to Battelle SOP No. 5-266, *Nutrient Sample Processing*. A 10-mL aliquot will be obtained from the particulate phosphorus filtrate. The sample will be passed through a Whatman 47-mm-diameter GF/F and collected in a polysulfon filtration flask. A clean 50-mL glass vial will be rinsed three times with filtrate then a 10-mL aliquot will be transferred by volumetric pipette into the vial. Samples will be stored frozen until analysis.

12.2.4 Particulate Carbon and Nitrogen

Samples for particulate carbon and particulate nitrogen will be processed according to Battelle SOP No. 5-266, *Nutrient Sample Processing*. The samples will be collected on precombusted 25-mm GF/F filters (nominal pore size 0.7 µm) using a vacuum-filter system. Each filter will be folded in half and placed in a labeled foil pouch and stored frozen until analysis. Samples will be processed in duplicate, but only one filter is analyzed, the second filter is for backup only or used as a laboratory duplicate.

12.2.5 Particulate Phosphorus

Samples for particulate phosphorus will be processed according to Battelle SOP No. 5-266, *Nutrient Sample Processing*. Samples will be collected on 47-mm GF/F using a vacuum-filter system. Each filter will be folded in half and placed in a labeled foil pouch and stored frozen until analysis. Samples will be processed in duplicate, but only one filter will be analyzed; the second filter will be for backup only or used as a laboratory duplicate.

12.2.6 Biogenic Silica

Samples for biogenic silica will be processed according to Battelle SOP No. 5-266, *Nutrient Sample Processing*. Samples will be collected on 47-mm-diameter Nuclepore membrane filters (0.4-µm pore size) using a vacuum-filter system. Each filter will be folded in quarters and placed in a labeled 50-mL plastic centrifuge tube and stored frozen until analysis. Samples will be processed in duplicate, but only one filter will be analyzed. The second filter is for duplicate analysis where applicable, or as backup.

12.2.7 Urea

Samples for urea will be processed according to Battelle SOP No. 5-266, *Nutrient Sample Processing*. A 40-mL aliquot will be obtained from the particulate phosphorus filtrate. Sample will be passed through a Whatman 47–mm–diameter GF/F and collected in a polysulfon filtration flask. A clean 60-ml polyethylene bottle will be rinsed three times with filtrate then filled with approximately 30 mL of filtrate. Samples will be stored frozen until analysis.

12.2.8 Chlorophyll *a* and Phaeophytin

Samples for chlorophyll *a*/phaeophytin determination will be processed according to Battelle SOP No. 5-265, *Extraction and Analysis of Chlorophyll* a *and Phaeophytin* a *in Seawater using a Turner* [Designs] *Model 10AU Fluorometer*. Samples for chlorophyll *a* analysis will be collected on Whatman 47-mm-diameter GF/F using a vacuum-filter system. A saturated solution of $MgCO_3$ will be added to the sample during filtration to aid retention and buffer the sample against low pH (which converts chlorophyll to phaeophytin). The filter will be stored frozen until analyzed.

12.2.9 Total Suspended Solids

Samples for total suspended solids (TSS) determination will be processed in a particulate free area according to Battelle SOP No. 5-053, *Suspended Particulate Matter Measurements (Total Suspended Solids [TSS])*. Using a vacuum-filter system, 500 mL of seawater will be passed through a precleaned and preweighed 0.4-μm pore size Nuclepore 47-mm-diameter membrane filter. Should the filtration rate slow substantially a lesser volume will be processed. The filter will then be rinsed three times with pH 8 deionized water to remove salt. Duplicate filters will be processed in parallel for each sample. Filters will be folded in quarters, placed in petri dishes, and stored at room temperature until returned to the laboratory.

12.2.10 Dissolved Oxygen (DO)

Samples for dissolved oxygen determination will be processed and analyzed shipboard and calculated using the procedures described in Battelle SOP No. 5-257, *Determination of Dissolved Oxygen by Modified Winkler Method*. Samples for DO analysis will be collected in 300-mL BOD bottles. Using a hose (about 50-cm long) attached to the nipple on the Rosette sampling bottle, fill the BOD bottle from the bottom up with a minimum of bubbles and turbulence. The BOD bottle will be placed in an overflow container that has a volume 3-4 times greater than the BOD bottle. The BOD bottle will be filled and allowed to overflow until the overflow container is full. After filling the BOD bottles, the DO samples will be fixed with manganese sulfate and alkali-iodide-azide as described in APHA *et al.* (1989). The samples will be stored in the dark for a minimum of 2 h and shaken and titrated within 24 h. The fixed oxygen samples will be titrated in the BOD bottle using a programmed Radiometer ABU91-21/TIM90-1 autotitrator with a precise potentiometric endpoint. These samples will be titrated either on board the vessel or onshore within 24 h of being fixed. Bottles will be kept dark until the samples are analyzed.

12.2.11 Respiration

Water will be collected in six 300–mL BOD bottles at each of three depths (surface, mid-depth, and bottom). Three bottles will be fixed immediately according to Battelle SOP No. 5-257, *Determination of Dissolved Oxygen by Modified Winkler Method* and used to determine initial DO concentration. Three bottles will be incubated in the dark, in temperature controlled incubators that are maintained to within 2°C of *in situ* temperature. The incubation will last from three to seven days, depending on the incubation

temperature (lower temperature, longer incubation). After the incubation period, the dark BOD bottles will be fixed for the determination of DO concentrations. These fixed samples will be analyzed within 24 h of being fixed.

12.2.12 Primary Productivity Analysis by ^{14}C

For the productivity stations, the mid-surface, mid–depth, and mid–bottom depths will be adjusted for 50%, 25%, and 10% light extinction while still getting the chlorophyll maximum, thus water for productivity are taken from the same bottles as the other analytes. From each of 5 depths at each productivity station, samples will be obtained by filtration through 300-µm-mesh screen (to remove large zooplankton) from the Rosette sampling bottle into opaque 1-L polyethylene bottles. The bottles will be rinsed twice prior to filling. The samples will be placed in a cooler and transferred to the URI laboratory within 5 hours of water sampling.

12.2.13 Whole-Water Phytoplankton

Water from the Rosette sampling bottle will be poured into a graduated cylinder that has been cut at the 850 mL mark. Before filling the cylinder, it is rinsed twice with water from the Rosette sampling bottle. The filled cylinder is then poured into a 1-L bottle containing 8mL of Utermöhl's solution preservative. The preserved samples are stored at ambient temperature and in the dark until analysis.

The Utermöhl's solution is prepared as described in Guillard (1973): 100 g potassium iodide, 50 g iodine, and 50 g sodium acetate each are dissolved incrementally in distilled water to a final volume of 1 L.

12.2.14 Screened and Rapid-Analysis Phytoplankton

For the screened and rapid analysis samples, a 4–L graduated cylinder is rinsed twice and filled with sample water from the Rosette sampling bottle. The water from the filled cylinder is passed through a 20-µm-mesh screen. Using a squeeze bottle that contains water that has passed through the 20-µm-mesh screen, water is squirted back through the screen to wash the retained plankton into a 1-L sample bottle containing 5 mL of Utermöhls solution. The plankton samples will be stored at ambient temperatures in the dark until analyzed by UMD. The rapid analysis sample will be transferred to UMD for immediate analysis.

12.2.15 Zooplankton

After conducting the net tow, the net is suspended with the net opening 7-9 feet above the deck. The suspended net is washed down from the outside of the net with running seawater. Excess water is drained through the netting. Again the lower part of the net is washed down from the outside of the net. This is repeated a couple of times until the net bottle is about ½ full and the netting is clear of material. The net bottle is removed from the end of the net and the retained water with material is transferred to a 1-L plastic jar. Using water from a squeeze bottle that was pre-screened with a 20-µm-mesh screen, any remaining material in the net bottle is washed into the plastic jar. Immediately, the sample will be preserved with enough buffered formalin to produce a 5-10% formalin to seawater solution. All zooplankton samples will be stored at ambient temperature in the dark until they are analyzed.

12.3 Laboratory Sample Processing and Analysis

12.3.1 Dissolved Inorganic Nutrients

Lambert and Oviatt (1986) described the analysis of dissolved inorganic nutrients. The filtrate concentrations of ammonium, nitrate, nitrite, silicate, and phosphate will be measured colorimetrically on a Technicon II Autoanalyzer. This instrument automates standard manual techniques for the analysis of nutrients. The analysis of ammonium will be based on the technique of Solorzano (1969) whereby absorbance of an indophenol blue complex is measured at 630 nm. Nitrite will be measured by the method of Bendschneider and Robinson (1952). The total of nitrate and nitrite is determined by reducing all nitrate in the sample to nitrite and analyzing for nitrite as above. The concentration of nitrate is obtained by difference. The reduction is accomplished using a cadmium column (Morris and Riley 1963). The analysis of phosphate will be based on the molybdate blue procedure of Murphy and Riley (1962). The colorimetric analysis of silicate will be based on that of Brewer and Riley (1966).

12.3.2 Dissolved Organic Carbon

CBL's Shimadzu 5000 Total Carbon Analyzer will be used to perform this analysis. This instrument uses an automated, high-temperature (680 °C) combustion technique where the sample is oxidized into carbon dioxide. A platinum catalyst greatly enhances this reaction. The carbon dioxide content is measured via a non-dispersive infrared detector (Menzel and Vaccaro 1964).

12.3.3 Total Dissolved Nitrogen and Phosphorus

CBL will use the Technicon Autoanalyzer II to perform this analysis according to the methods of D'Elia *et al.* (1997) and Valderrama (1981). This method is a persulfate oxidation technique for nitrogen and phosphorus where, under alkaline conditions, nitrate is the sole nitrogen product and phosphate is the sole phosphorus product. Dissolved organic P is the difference between total dissolved P and Phosphate. Dissolved organic N is the difference between total dissolved N and dissolved inorganic nitrogen components.

12.3.4 Particulate Carbon and Nitrogen

The analysis, performed on CBL's Exeter Analytical Model CE-440 Elemental Analyzer, is a high temperature combustion where the combustion products - water vapor, carbon dioxide and nitrogen gas - are analyzed via a series of thermal conductivity cells and compared to a known standard (Menzel and Vaccaro 1964 and EPA Method 440.0 [March 1997]). This analysis does not distinguish between particulate organic and particulate inorganic components of a sample. In other studies, CBL has determined that the carbon and nitrogen particulate component is almost entirely organic in natural waters of such areas as Chesapeake Bay, Long Island Sound, and New York Bight.

12.3.5 Particulate Phosphorus

The frozen filter will be placed in a 12-mL centrifuge tube and 2 mL of 0.017 M $MgSO_4$ will be added. The tube will be placed in a drying oven (95 °C) until dry. The tube will be tightly capped with pre-ashed foil, and subsequently ashed for 2 h at 450 °C. Once cool, 5 mL of 0.2 M HCl will be added and the tube tightly capped. The tube will be placed in an 80 °C drying oven for one-half hour and cooled to room temperature. 5 mL of deionized water will be added to the tube. The sample will be analyzed by using a Technicon Autoanalyzer II (Solorzano and Sharp 1980).

12.3.6 Biogenic Silica

Biogenic silica will be analyzed according to the method developed by Paasche (1973). This is an extraction/digestion technique using NaOH in a 100°C water bath followed by analysis of silicate in the extract by a Technicon Autoanalyzer II System.

12.3.7 Urea

Urea samples will be analyzed by hand according to the methods of Price and Harrison (1987). The method is a colorometric analysis of the reaction of urea with diacetylmonoxime in an acid solution. For very large batch sizes, this analysis may be automated using an Alpchem autoanalyzer rather than the Shimadzu UV-Visible (Model UV1601) Spectrophotometer.

12.3.8 Chlorophyll *a* and Phaeophytin

Samples for chlorophyll *a*/phaeophytin will be processed according to Arar and Collins (1992) and Battelle SOP 5-265, *Extraction and Analysis of Chlorophyll a and Phaeophytin a in Seawater Using a Turner Designs Model 10AU Fluorometer*. Samples will be processed in subdued light and stored at -20º C between handling steps. The chlorophyll *a*/phaeophytin will be extracted from the cells retained on the GF/F filter, by mechanical grinding followed by a 2-4 hour steep in 90% acetone at -20º C. The sample will then be centrifuged and the extract analyzed using a Turner Designs Fluorometer. Two drops of 1 N HCl will be added to the extract and the extract remeasured to determine phaeophytin concentrations. The grinding apparatus and cuvette are rinsed between samples by using 90% acetone.

12.3.9 Total Suspended Solids

Laboratory analysis for Total Suspended Solids will be performed according to Battelle SOP 5-053, *Suspended Particulate Matter Measurements (Total Suspended Solids [TSS]).* Filters will be dried in a class 100 clean bench for at least 48 hours. Sample-laden filters will then be reweighed. TSS will be calculated as the net filter weight, and will be reported as the mean of the duplicate samples.

12.3.10 Dissolved Oxygen

After filling all of the BOD bottles from the Rosette sampling bottles, the DO samples will be fixed with manganese hydroxide and alkali-iodide as described by Oudot *et al.* (1988) and APHA *et al*. (1989) and documented in Battelle SOP No. 5-257, *Determination of Dissolved Oxygen Concentration in Water by Modified Winkler Method using the Radiometer TitraLab*. Fixed oxygen samples will be titrated in the bottle using a programmed Radiometer ABU91-21/TIM90-1 autotitrator with a precise potentiometric endpoint. Within 24 h of being fixed, these samples will be titrated either on board the vessel or onshore. The concentration of DO in units of (mg O_2 L^{-1}) will be determined using the following equation:

$$DO = \frac{A\ F}{V}$$

where A = Volume of titrant in (mL)
V = Volume of DO (mL)
F = Factor based on standardization of thiosulfate titrant against a potassium iodate standard of known molarity.

12.3.11 Respiration

The rate of oxygen consumption will be calculated using the method described by Strickland and Parsons (1972) and Battelle SOP No. 5-257. The corresponding initial DO samples will be used in the calculation. The samples not immediately fixed for DO analysis will be processed as described in Section 12.2.11. The net respiration (NETR) in units of mg O_2 L^{-1} h^{-1} will be determined using the equation below. The result is multiplied by 1000/32 to derive the rate of 0_2 decline in μM/h.

$$NETR = \frac{(DO_{IB} - DO_{DB})}{T}$$

where DO_{1B} = Initial DO concentration in mg O_2 L^{-1}
DO_{DB} = Dark Bottle DO concentration in mg O_2 L^{-1} after incubation
T = Incubation time in hours

12.3.12 Primary Production by ^{14}C

Under subdued green light, each depth will be processed separately starting with the surface water sample. Each sample will be mixed thoroughly and then poured into a repipette set to deliver 5 mL. The repipette will be rinsed twice with sample prior to use. The delivery tip of the repipette will be flushed three times and 5 mL of sample will be pipetted into 20 mL borosilicate vials. A total of 16 bottles (14-16 light bottles, 2 dark bottles) will be filled for each depth. These vials will be incubated in a light and temperature controlled incubator. Light bottles from each depth will be incubated at 14 to 16 light intensities (250 w Tungsten-halogen lamps attenuated with neutral density filters) and all bottles will be incubated within 2ºC of the *in situ* temperature.

The 5 mL samples will be incubated with 100 μL of 10 μCi/mLl (1 μCi for 5 mL sample) Carbon-14 (^{14}C) stock solution. All vials will then be placed in the incubator for two hours. Time and temperature will be recorded at the start and end of the incubation period. The light intensity within the incubator will be measured before and after the incubation period. Temperature will be constantly monitored throughout the incubation period and the location of each vial in the incubator will be recorded. Upon removal from the incubator, 100 μL of 0.05N HCl, will be added to each vial. Vials will remain loosely capped while shaken overnight. The following morning 15 mL Ecolume will be added to each vial, which will again be loosely capped and shaken overnight. Two days following the cruise, vials will be tightly capped and placed on the Beckman LS 3801 to be counted.

Calculation of Primary Production. Volume-specific primary production will be calculated using equations similar to that of Strickland and Parsons (1972) as follows:

$$P(i) = \frac{1.05(DPM(i))DIC}{A_{sp}T}$$

$$P(d) = \frac{1.05(DPM(d))DIC}{A_{sp}T}$$

$$A_{sp} = DMP(sa) - DPM(back)$$

where:

$P(i)$ = primary production rate at light intensity i (µgC $L^{-1}h^{-1}$ or mgC $m^{-3}h^{-1}$)
$P(d)$ = dark production, (µgC $L^{-1}h^{-1}$ or mgC $m^{-3}h^{-1}$)
$DPM(i)$ = dpm in sample incubated at light intensity i
$DPM(d)$ = dpm in dark incubated sample
$DPM(back)$ = background dpm in vial containing only scintillation cocktail
$DPM(sa)$ = specific activity added to incubation samples (DPM)
T = incubation time (h)
DIC = concentration of dissolved inorganic carbon (µg/mL)

Table 13 shows the frequency that primary productivity measurements and calculations are performed per vial, depth, station, and survey.

Table 13. Measurement frequency for variables involved in calculation of primary production.

Measurement/ Calculation	Vial	Depth	Station	Survey
DPM(i)	✔	✔	✔	✔
P(i)	✔	✔	✔	✔
DIC		✔	✔	✔
P(d)		✔	✔	✔
DPM(d)			✔	✔
Asp			✔	✔
T			✔	✔
DPM (sa)			✔	✔
DPM(back)			✔	✔

P–I curves. For each of the 5 depths for each photosynthesis station a P–I curve will be obtained from the data P(I) = P(i)-P(d) vs. the irradiance (I, µE $m^{-2}s^{-1}$) to which the incubating sample is exposed. The P-I curves will be fit via one of two possible models, depending upon whether or not significant photo-inhibition occurs. In cases where photoinhibition is evident the model of Platt *et al.* (1980) will be fit (SAS 1985) to obtain the theoretical maximum production, and terms for light-dependent rise in production and degree of photoinhibition:

$$P(I) = P_{sb}(1-e^{-a})e^{-b}$$

where:

$P(I)$ = primary production at irradiance I, corrected for dark fixation (P(i)-P(d))
P_{sb} = theoretical maximum production without photoinhibition
$a = \alpha I / P_{sb}$ and α is the initial slope, the light-dependent rise in production
$b = \beta I / P_{sb}$ and β is a term relaying the degree of photoinhibition

If ß is not significantly different from zero, an alternative model of Webb *et al.* (1974) will be similarly fit to obtain the maximum production and the term for light-dependent rise in production:

$$P(I) = P_{\max}(1 - e^{-a'})$$

where:

$P(I)$ = primary production at irradiance I corrected for dark fixation (P(i)-P(d))

$P_{\max}$ = light saturated maximum production

$a' = \alpha I / P_{\max}$ and α is the initial slope the light-dependent rise in production

Light vs. Depth Profiles. To obtain a numerical representation of the light field throughout the water column averaged CTD light profiles (0.5 m intervals) will be fit (SAS 1985) to an empirical sum of exponentials equation of the form:

$$I_Z = A_1 e^{-a_1 Z} + A_2 e^{-a_2 Z} + ...$$

which is an expansion of the standard irradiance vs. depth equation:

$$I_Z = I_0 e^{-kZ}$$

where:

I_Z = light irradiance at depth Z

I_0 = incident irradiance ($Z = 0$)

k = extinction coefficient

A_1, A_2 . . . = factors relating to incident irradiance ($I_0 = A_1 + A_2 + ...$)

a_1, a_2 . . . = coefficients relating to the extinction coefficient ($k = a_1 + a_2 + ...$)

The expanded equation will be used in most instances as spectral shifts, pigment layering and other factors result in deviation from the idealized standard irradiance vs. depth equation. The simplest form of the expanded equation will be implemented to adequately model the light field, which in the large majority of cases will be the sum of two exponentials.

Daily Incident Light Field. During normal CTD hydrocasts the incident light field is routinely measured via a deck light sensor at high temporal resolution. The average incident light intensity will be determined for each of the CTD casts to provide, over the course of the photoperiod (12-hr period centered upon solar noon), a well resolved irradiance time series consisting of 12-17 data points. A 48-point time series (every 15 min) of incident will be obtained form these data by linear interpolation. A similar time series of light data is collected at Deer Island, and will be used as the photoperiod incident light (I_o) time series described below.

Calculation of Daily Primary Production. Given the best fit parameters (P_{sb} or P_{max}, α, β) of the P-I curves obtained for each of the five sampling depths, the in situ light intensity (*i.e.*, I_z) at each depth determined from the sum of exponential fits on the in situ light field, and the photoperiod incident light (I_0) time series, it will be possible to compute daily volumetric production for each depth. To do this at a given depth, hourly production is determined for the in situ light intensity computed for each 15 min interval of the photoperiod, using the appropriate P-I parameters and in situ irradiance. Daily production ($\mu gC\ L^{-1}\ d^{-1}$) is obtained by integration of the determined activity throughout the 12-hour photoperiod. An advantage of this approach is that seasonal changes in photoperiod length are automatically

incorporated into the integral computation. For example, during winter months computed early morning and late afternoon production contributes minimally to whole day production, whereas during summer months the relative contribution during these hours is more significant. The investigator does not have to decide which factor to employ when converting hourly production to daily production. The primary assumption of the approach is that the P-I relationship obtained at the time of sample procurement (towards the middle of the photoperiod) is representative of the majority of production occurring during the photoperiod, which should be the case.

Calculation of Daily Areal Production. Areal production (mgC $m^{-2}d^{-1}$) will be obtained by trapezoidal integration of daily volumetric production vs. depth down to the 1% light level.

Calculation of Chlorophyll-Specific Parameters. Chlorophyll-specific measures of the various parameters (including the P-I parameters) will be determined by dividing by the appropriate chlorophyll term obtained from independent measurements.

12.3.13 Whole-Water Phytoplankton

At the laboratory, Utermöhl's-preserved whole seawater samples will be prepared for analysis by concentrating the sample by gravitational settling as described by Borkman (1994), Borkman *et al.* (1993), Turner *et al.* (1995). The method is similar to the methods of Hasle (1959), Iriarte and Fryxell (1995), and Sukhanova (1978). Samples will be settled in graduated cylinders with no more than a 5-to-1 height-to-width ratio.
Phytoplankton abundance is estimated by counting phytoplankton cells in a 1-mL capacity Sedgwick-Rafter chamber. Phytoplankton cells will be observed, counted, and identified in a two-stage counting protocol utilizing 250× and 500× magnifications. In this protocol, the Sedgwick-Rafter chamber is divided into equal, horizontal paths or strips and cells are enumerated as one moves across randomly selected strips. Small cells (*e.g.*, microflagellates, *Cryptomonas*) and larger forms will be counted at 500×, with counting of small cells proceeding at 500× until the end of the path in which the 400-entities minimum tally is reached. The analysis will continue at 250×, where paths of the Sedgwick-Rafter chamber will be examined until at least 75 entities (unicellular forms, colonies, or chains) of each of the three most abundant taxa are observed.

The two-step counting protocol allows for improved precision in estimating abundances of small (<10μm greatest axial linear dimension) and larger phytoplankton forms. Counting large numbers of small forms at 500× increases the precision of the estimated abundances of these forms (see Section 11 for a discussion of precision). The counts at 250× allow for the examination of a larger volume of the sample, thereby increasing the likelihood of encountering larger, less abundant (or rare) forms. During the 250× analysis, the 500× objective can be used as needed to resolve key taxonomic characters.

Phytoplankton abundance is calculated by dividing the number of cells counted by the volume examined in Sedgwick-Rafter chamber. Calculation of abundance also accounts for the concentration factor (nominally 16:1) used in the settling process. Final abundance estimates will be reported as units of 10^6 cells per liter.

12.3.14 Screened Phytoplankton (Dinoflagellates)

A taxonomist will identify and count the following target organisms. Additional taxa may be noted at the discretion of the taxonomist.

Alexandrium tamarense

Ceratium sp.
Dinophysis sp.
Gymnodinium sp.
Gyrodinium sp.
Heterocapsa triquetra
Prorocentrum sp.
Protoperidinium sp.
Heterosigma akashiwo (formerly *Olisthodiscus luteus*)
Phaeocystis pouchetii

Samples will be concentrated by gravimetric sedimentation to final volumes of between 5 and 25 mL; the final volume will depend upon the amounts of particulates present in the sample, which is a subjective judgment made by the analyst prior to sample sedimentation. The final volume settled will be selected to minimize interference by detritus and to maximize the numbers of target organisms. A typical sample will be settled to final volumes of 5 to 10 mL. A Sedgwick-Rafter cell will be filled with 1 mL of concentrated sample and all target algae (listed above) will be identified and counted until either 400 cells or all cells in the entire Sedgwick-Rafter cell are counted, whichever comes first.

12.3.15 Rapid-Analysis Samples

The screened, rapid-analysis samples will be examined for qualitative impression of the dominant taxa and specific harmful or toxic alga (*i.e., Alexandrium tamarense*, *Phaeocystis* sp., *Pseudo-nitzschia*). Within six days of sample receipt at the counting laboratory, an aliquot of this sample will be qualitatively analyzed using the Sedgwick-Rafter counting cell and viewed through an Olympus BH-2 compound microscope (phase-contract Optics) to quickly verify the presence or absence of nuisance species. The analysis will also produce a qualitative impression of the types and abundance of dominant taxa.

12.3.16 Zooplankton

Upon return to shore, samples for zooplankton are transferred to 70% ethanol solutions to prevent inhalation of formalin fumes during counting. Samples are reduced to aliquots of at least 300 animals with a Folsom plankton splitter, and animals are counted under a dissecting microscope and identified to the lowest possible taxon. In most cases, this will be to species; adult copepods will be additionally characterized by sex. Counts of all copepodite stages of a given copepod genus will be combined. Copepod nauplii will not be identified to genus or species because nauplii species cannot be reliably identified to those levels by using a dissecting microscope. Meroplankters cannot be identified to genus or species in most cases, and such organisms will be identified to the lowest reliable taxon, such as barnacle nauplii, fish eggs, or gastropod veligers.

Concentrations of total zooplankton and all identified taxa are calculated based on the number of animals counted, divided by the volume of water filtered by the net, multiplied by the aliquot concentration factor.

13.0 SAMPLE CUSTODY

Samples collected in the field will be identified by a unique eight character *Sample ID* which is a concatenation of a five character *Event ID* and a three-character hexadecimal number (*Sample_Marker*). The *Sample ID* will identify the water collected in the Rosette sampling bottles from a certain depth during a particular station on the specified survey. The five character *Event ID* will be unique to each survey, such as WF987, with “WF” indicating that it is a farfield water column survey, “98” indicating the survey year, and “7” signifying the seventh survey of the year (for surveys higher than 9, letters are

used where A and B are equal to 10 and 11, respectively). The *Sample_Marker* is a non-repeating (within a survey) number generated by the NAVSAM software during the closing of a set of Rosette sampling bottles at one depth or at completion of the vertical net tow.

Each portion of a sample separated for analytical purposes will be assigned a unique *Bottle ID*, composed of the eight–character *Sample ID* plus a 3–character suffix designating the nature and replicate number. For example, "IN2" indicates that the subsample is the second replicate for Dissolved Inorganic Nutrient analyses (see Table 13 for two-letter codes). Information relating to each sub-sample will then be recorded in the *Bottle* table in the EM&MS database.

Before the field surveys are initiated, a table of all samples to be collected will be prepared. Appendix A contains examples of tables for nearfield (Table A-1) and farfield (Table A-2) surveys. The information in those tables are used to generate the station logs (Figure 11) and to generate a planned-bottle table (Table 14) for use during the surveys.

Table 14. Analysis Codes used in *Bottle ID*

Analysis Codes	Description	Laboratory
AP	Primary productivity	URI
BS	Biogenic silica	CBL
CH	Chlorophyll	Battelle
DO	Dissolved oxygen	Battelle
IC	Dissolved inorganic carbon	URI
IN	Dissolved inorganic nutrients	URI
NP	Total dissolved nitrogen and phosphorous	CBL
OC	Dissolved organic carbon	CBL
PC	Particulate carbon and nitrogen	CBL
PP	Particulate phosphate	URI
RE	Respiration	Battelle
RP	Rapid analysis phytoplankton	UMD
SE	Secchi	Battelle
SW	Screened water phytoplankton	UMD
TS	Total suspended solids	Battelle
UR	Urea	MBL
WW	Whole water phytoplankton	UMD
ZO	Zooplankton	UMD

Table 15. Planned Bottle Table Structure

Field Name	Description
Station ID	Station ID from Tables A1 and A2
Group ID	Group ID based on survey type and sampling depth (*i.e.*, F3 is Farfield station sample taken at mid-depth)
Analysis ID	Two-letter analysis code list in Table 13
Rep Number	Replicate number (1 through 6)

The scientific crew member operating the data collection system will fill out the station log at each station. These logs will be put into a survey notebook prior to the survey. The log includes fields for entering pertinent information about each station, such as time on station, bottom depth, weather observations, and general comments. During the hydrocast CTD data will be logged and stored electronically on the computer's hard disk. When Rosette sampling bottles are closed, the operator will enter the Group ID and mark an event into the CTD data file and the survey electronic log.

At the end of a profile, sample marker information is joined with the planned bottle table to generate station log label and bottle labels. The bottle label will include the Bottle ID in text and barcode (3 of 9 format), the station, date, time, latitude/longitude, depth for the sample, and analysis code. The data files saved by the software will also be used later as entry into the SAMPLE, STATION, PROFILE, BOTTLE, EVENT, and STATION_TYPE tables of the EM&MS database (see Section 15 for more information).

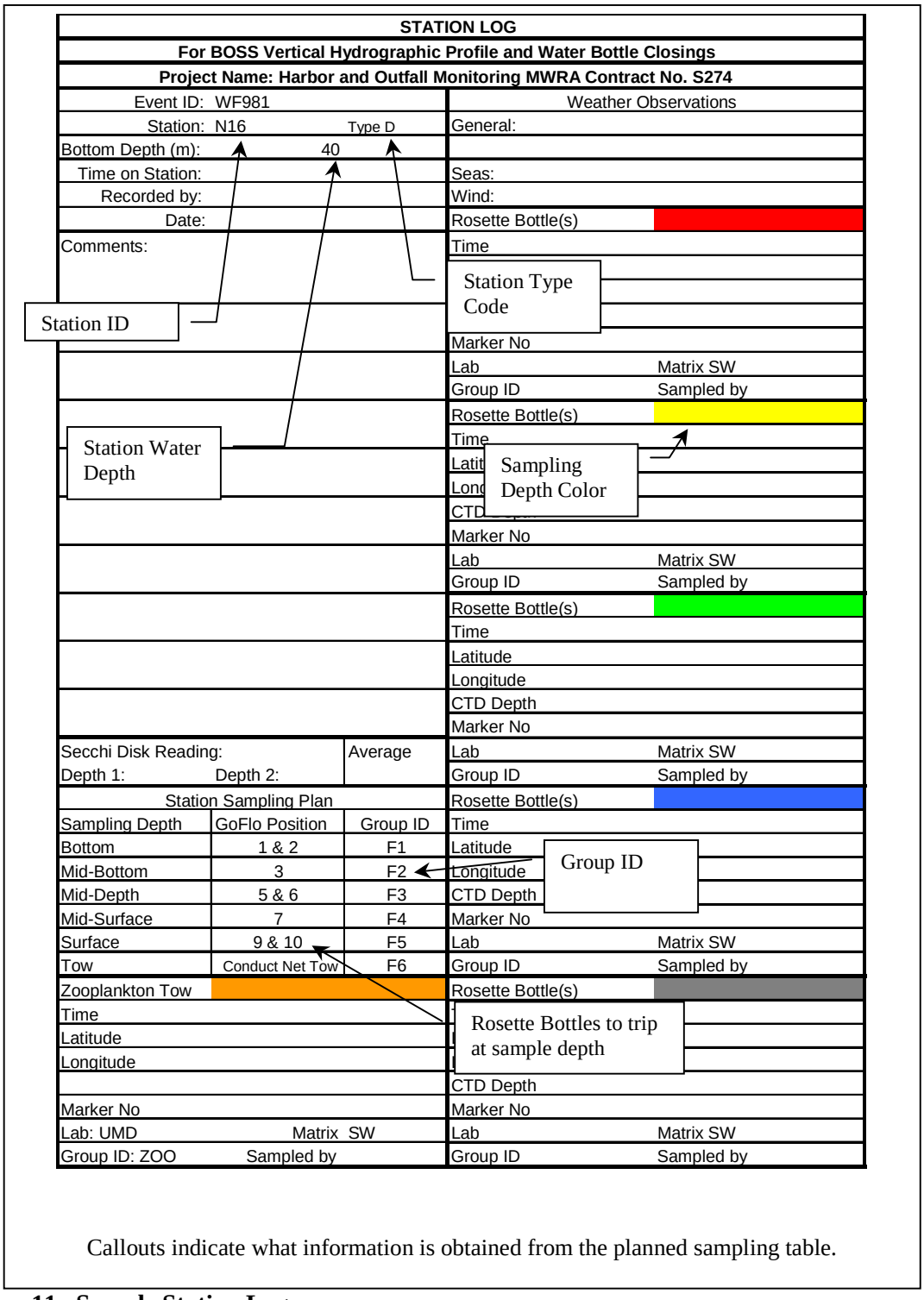
After all of the samples for a survey are collected, chain-of-custody forms (Figures 12 and 13) for each type of sample will be generated. Using the chain-of-custody forms, the samples will be inventoried before the samples are transferred. When the custody of samples is transferred, the custody form will be signed by both the staff member that relinquishes custody and the staff member assuming custody for the samples. The relinquishing staff member will retain a photocopy of the signed COC. After the analysis is completed, the original (signed) COC will be given to the Laboratory Manager to be placed in the project files.

13.1 Custody of Electronic Data

Field custody of electronic data will be the responsibility of the survey chief scientist. This person will be identified for each survey. The field custody of the electronic data consists of creating floppy-disk backups of all electronic data generated each day. Each floppy disk label will include a survey ID, date, name of person creating the backup files, and a disk number. When the equipment is returned to Battelle, a second complete backup labeled as "Set 2", will be generated on floppy disks. The backup will be in the custody of Mr. Albro. The survey chief scientist maintains the original.

Battelle, CBL, URI, MBL, and UMD will produce electronic data generated under this task. At Battelle, the electronic files for chlorophyll a, TSS, and DO data will remain in the custody of the Task Leader (Mr. Scott Macomber) until all analyses are completed and data have gone through the Battelle Quality Assurance Unit. The data will be entered into a loading application that contains the data integrity checks for the EM&MS. The data from the loading application will be subjected to QA audit for analytical processing. Two copies of each type of electronic file will be made. Set 1 will remain in custody of the Task Leader in the Task notebook. Set 2 will be transferred the HOM3 Database Manager for entry into the MWRA database.

Electronic data will remain in the custody of laboratory managers and custodians [Mr. Carl Zimmermann (CBL), Dr. Jefferson Turner (UMD), Dr. Candace Oviatt (URI), Ms. Jane Tucker (MBL)] until an independent QA audit has been completed. Once the data have passed the independent laboratory QA audit, three copies of each type of electronic file will be made. Set 1 will remain in the custody of the subcontractor custodians and Sets 2 and 3 will be sent to the Battelle. Set 2 will be stored in the Task notebook and Set 3 will be given to the Battelle Database Manager for entry into the MWRA database.



Callouts indicate what information is obtained from the planned sampling table.

Figure 11. Sample Station Log

CHAIN-OF-CUSTODY RECORD		
Zooplankton		
MWRA HOM-3 - Water Column Monitoring		
REMEMBER TO RECORD ALL LEADING ZEROS WHEN RECORDING FLOW METER READING	Battelle Ocean Sciences 397 Washington Street Duxbury, MA 02332	
Label	Initial Flow meter Reading:	Date:
	Net Tow Time in seconds:	Time:
	Final Flow meter Reading:	Initials:
	Formaldehyde Added (mls):	
	Comments:	
Label	Initial Flow meter Reading:	Date:
	Net Tow Time in seconds:	Time:
	Final Flow meter Reading:	Initials:
	Formaldehyde Added (mls):	
	Comments:	
Label	Initial Flow meter Reading:	Date:
	Net Tow Time in seconds:	Time:
	Final Flow meter Reading:	Initials:
	Formaldehyde Added (mls):	
	Comments:	
Label	Initial Flow meter Reading:	Date:
	Net Tow Time in seconds:	Time:
	Final Flow meter Reading:	Initials:
	Formaldehyde Added (mls):	
	Comments:	
Label	Initial Flow meter Reading:	Date:
	Net Tow Time in seconds:	Time:
	Final Flow meter Reading:	Initials:
	Formaldehyde Added (mls):	
	Comments:	
Label	Initial Flow meter Reading:	Date:
	Net Tow Time in seconds:	Time:
	Final Flow meter Reading:	Initials:
	Formaldehyde Added (mls):	
	Comments:	
Relinquished By/Date/Time/Company	Received By/Date/Time/Company	
Please return original of this completed Chain-of-Custody form to Heather Trulli - Battelle		

Figure 12. Example of a Zooplankton Chain-of-Custody Form

MWRA Harbor and Outfall Monitoring Program

Contract No. S274

Chain-of-Custody Form

Today's Date : 6/9/98 9:09:07 AM

Chain-of-Custody # : WN985-BS-0146
Survey ID : WN985
Analysis ID : BS
Analysis Description : Biogenic silica

Laboratory : Chesapeake Biological Laboratory
Nutrient Analytical Services
Box 38
Solomons MD 20688
Dr. Carl Zimmerman
410-326-7252 (Phone) 410-326-7209 (Fax)

Bottle ID :	Bottle ID :	Sampling Date :	Station ID :	Ck 1	Ck 2	Ck 3	Ck 4
[redacted]	WN98500BBS1	5/1/98 8:13:44 AM	N04	☐	☐	☐	☐
[redacted]	WN98500BBS2	5/1/98 8:13:44 AM	N04	☐	☐	☐	☐
[redacted]	WN98500DBS1	5/1/98 8:16:38 AM	N04	☐	☐	☐	☐
[redacted]	WN98500DBS2	5/1/98 8:16:38 AM	N04	☐	☐	☐	☐
[redacted]	WN98500FBS1	5/1/98 8:18:46 AM	N04	☐	☐	☐	☐
[redacted]	WN98500FBS2	5/1/98 8:18:46 AM	N04	☐	☐	☐	☐
[redacted]	WN985030BS1	5/1/98 10:22:29 AM	N18	☐	☐	☐	☐
[redacted]	WN985030BS2	5/1/98 10:22:29 AM	N18	☐	☐	☐	☐
[redacted]	WN985032BS1	5/1/98 10:25:17 AM	N18	☐	☐	☐	☐
[redacted]	WN985032BS2	5/1/98 10:25:17 AM	N18	☐	☐	☐	☐
[redacted]	WN985034BS1	5/1/98 10:27:04 AM	N18	☐	☐	☐	☐
[redacted]	WN985034BS2	5/1/98 10:27:04 AM	N18	☐	☐	☐	☐
[redacted]	WN985059BS1	5/1/98 12:11:34 PM	N10	☐	☐	☐	☐
[redacted]	WN985059BS2	5/1/98 12:11:34 PM	N10	☐	☐	☐	☐
[redacted]	WN98505BBS1	5/1/98 12:13:53 PM	N10	☐	☐	☐	☐
[redacted]	WN98505BBS2	5/1/98 12:13:53 PM	N10	☐	☐	☐	☐
[redacted]	WN98505DBS1	5/1/98 12:16:18 PM	N10	☐	☐	☐	☐
[redacted]	WN98505DBS2	5/1/98 12:16:18 PM	N10	☐	☐	☐	☐

Shipping Condition - Room Temperature: _____ Cold(ice): _____ Frozen(dry ice): _____
Received Condition - Room Temperature: _____ Cold(ice): _____ Frozen(dry ice): _____

Relinquished By / Date / Time / Company / Transport-Airbill #	Received By / Date / Time / Company

Figure 13. Example of Water Chemistry Chain-of-Custody Form

13.2 Custody of Water Samples

During field collection, COC forms will be completed and labels will be affixed to the sample containers, thereby creating a link between the sample and data recorded on the COC form. The COC forms will have the same alphanumeric code as the corresponding label on the sample container, ensuring the tracking of sample location and the status.

The samples will remain in the custody of the Field Sample Custodian (designated by the Chief Scientist for each survey) while in the field. COC forms will accompany the samples when transferred from the field to the laboratory. All samples will be distributed to the appropriate laboratory personnel by hand or by Federal Express. When samples arrive at each laboratory, custody will be relinquished to the Laboratory Custodian. Upon receipt of the samples at Battelle or its subcontractors, the Sample Custodian will examine the samples, verify that sample-specific information recorded on the COC is accurate and that the sample integrity is uncompromised, log the samples into the laboratory tracking system, complete the custody forms, and sign the COC form so that transfer of custody of the samples is complete. Any discrepancies between sample labels and transmittal forms, and unusual events or deviations from the project CW/QAPP will be documented in detail on the COC and the Task Leader and Project Manager notified. The original COC forms will be submitted to the Battelle Laboratory Manager and maintained in the MWRA project files. Sample numbers that include the complete field ID number will be used to track the samples through the laboratory. Alternately, unique laboratory IDs may be assigned by each laboratory for use during their sample analyses, but the data will be reported to the database by using the field-generated sample number.

During sample processing, a suite of samples for each station will be generated. To identify the suite of samples, a protocol coding system has been developed specifically for the MWRA project. Table 12 shows the protocol codes and parameters to be analyzed for each protocol code. Sample processing may be documented on a project-specific processing form.

Samples that have been analyzed and have passed their holding times will be discarded. No samples will be archived.

14.0 CALIBRATION PROCEDURES AND PREVENTIVE MAINTENANCE

Logs of maintenance and repairs made to instruments will be stored in the instrument files maintained by Battelle and by the subcontractors. Maintenance of and repairs to instruments will be in accordance with manufacturers' manuals. Any deviations to this policy will be noted.

14.1 Hydrographic Profiling Equipment

14.1.1 Pressure (Depth) Sensor

At the beginning of each survey, the software offset of the Sea-Bird SBE-9 CTD depth sensor is set to read zero when the sensor is on deck. The offset is entered into the equipment setup file. The offset of the pressure reading is affected by the atmospheric pressure.

14.1.2 Temperature and Conductivity

The software gain and offset of the temperature and conductivity sensors are calibrated annually at the factory and the factory calibration settings are not changed. A review of the calibration coefficients for

the CTDs shows that they are quite stable from year to year. Based on the annual calibrations of the Sea-Bird CTD, the annual drifts are 0.002 °C for temperature, 0.0396 mS/m for conductivity, 0.036 PSU for salinity, and 0.028 for Sigma-T. Based on the annual calibrations of the Ocean Sensors CTD, the drifts are 0.018 °C for temperature, 0.042 mS/m for conductivity, 0.055 PSU for salinity, and 0.046 for Sigma-T.

14.1.3 *In Situ* Dissolved Oxygen

The software gain and offset of the dissolved oxygen sensors will be calibrated annually at SeaBird and the calibration settings will not be changed thereafter. The DO values determined by the sensor will be corrected for each survey based on a comparison with discrete water samples analyzed by titration. The DO data from the sensor (based on factory calibration settings) will be entered into a MS Excel spreadsheet along with the corresponding bottle samples data. Using the built-in linear regression analysis tool, the correction slope and intercept will be determined. The regression will be based on the following equation:

DO conc. (from sensor) = slope x DO conc. (bottle value) + intercept

To correct the CTD values in the database, the following equation will be used:

Corrected sensor DO conc. = [DO conc. (from sensor) - intercept]/slope

14.1.4 Transmissometer

The transmissometer is calibrated annually in the laboratory at Battelle. This calibration consists of obtaining voltage readings under the three following conditions:

V_o = voltage when the light path is blocked
V_a = voltage in air
V_w = voltage in distilled water.

Beam attenuation for the 20-cm path length is calculated using the following equation:

$$c = A - 5 \ln (V_m - V_o)$$

where c = beam attenuation
A = offset coefficient
V_m = measured *in situ* voltage.

Knowing that the beam attenuation of clear distilled water is 0.364/m, the value of *A* is calculated as follows:

$$A = 0.364 + 5 \ln (V_w - V_o).$$

A review of the calibration coefficients for the transmissometer shows that it is quite stable from year to year. The drift of the transmissometer is dependent on the amount of time it is operated. For example, in 1992 the transmissometer drift was approximately 0.01/m after 288h of operation.

To check that the transmissometer is working properly, it will be checked each survey day by checking blocked (more than 40/m) and unobstructed (less than 0.5/m) readings in air using the BOSS program display. After each cast, the optics of the transmissometer will be rinsed with deionized water.

14.1.5 *In Situ* Chlorophyll *a* Fluorometer

Based on manufacturer's recommendations, the software gain and offset of the fluorometer are set annually. The fluorometer data, displayed with the NAVSAM program, will approach 0.0 μg/L when the instrument is on deck. As daily maintenance, the fluorometer will be rinsed with deionized seawater. During farfield surveys, the instrument will be turned off between stations to prevent flash-lamp degradation. The calculated readings will be corrected in the same manner as described for the DO sensor above, using the measured chlorophyll *a* data from discrete bottle samples to develop a linear regression and correction slope and intercept. In this case, the "bottle value" will be taken as the chlorophyll concentration plus the phaeophytin concentration reduced by a factor (1.7) to account for its lower fluorescence efficiency.

The regression will be based on the following equation:

Chg. conc. (from sensor) = slope *x* (Ch conc. (bottle value) + intercept

To correct CTD value in the database, use the following equation:

Corrected sensor Ch conc. = (Ch conc. (from sensor) – intercept)/slope

The calibration will be acceptable if the significance of F (confidence interval) is $\leq$0.05, and will be qualified as failed if the significance is >0.05.

14.1.6 Irradiance Profiling and On-deck Sensors

The QSP200L Biospherical irradiance sensor is interfaced to the BOSS system via the CTD and is used to measure photosynthetically active radiation underwater. The QSR240 is used to measure surface solar irradiance, and is interfaced to the BOSS system via the system's analog-to-digital converter. Both sensors are annually calibrated at the factory. On a clear day at local noon, the surface solar irradiance as measured by the QSR240 should be 2000-3000 $\mu Em^{-2}s^{-1}$. The same measurement on deck using the underwater sensor (QSP200L) should be 3500-4000 $\mu Em^{-2}s^{-1}$. The difference in the readings is caused by field-of-view differences and a correction factor applied to the underwater sensor to account for its lower collection efficiency when immersed.

Before each cast, the Seasoft software acquires readings from the sensors while the underwater unit is on deck. This information will be saved in a raw data file. The on-deck readings will be compared to see that both instruments are operating correctly. The sensors will be cleaned daily with a non-abrasive cloth and a solution of dish soap and water.

14.2 Navigation Equipment

Once the 12V DC-power supply for the Northstar DGPS navigation system has been switched on, there is typically no other setup interaction necessary between the NAVSAM operator and the navigation system. The GPS will also conduct an automatic self-test. Once the DGPS has acquired at least one satellite, the green LED on the front panel will start flashing. When the DGPS has acquired at least three satellites to give a correct position, the green LED will remain lighted constantly. The DGPS will display a latitude-longitude (L/L) position once the system has acquired an acceptable fix. The DGPS system provides guaranteed position accuracy of 10 meters 95% of the time.

Position calibration will be performed twice per day as follows:

1. An absolute position is obtained from published charts with a position accuracy approaching 2 sec (approx. 40 m).

2. The NAVSAM program is set to calibration-navigation mode.

3. Thirty fixes are obtained by the program, averaged, and then compared to the absolute position entered by the operator.

4. If a printer is connected to the system, a printout of the calibration is obtained. Otherwise, the data are manually entered into the first or last station log for that day.

14.3 Rosette Sampling Bottles

The Rosette sampling bottles are maintained by conducting annual functional checkouts including replacing worn, damaged components. During the surveys, the bottles are closed between stations. Just before arriving at a station, the bottle valves are attached to the Rosette mechanism, which holds the valves in an opened position. The bottles are "cleaned" during the downcast by the flushing of sample water through the bottles. The bottles are closed in the upcast.

14.4 Laboratory Instruments

Calibration procedures for laboratory instruments are summarized in Table 15. All laboratory calibration records will be reviewed by analysis task leaders and maintained in laboratory notebooks.

Table 16. Calibration Procedures for Laboratory Instruments

Parameter	Instrument Type	Initial Calibration			Continuing Calibration		Corrective Action
		No. Stds	Acceptance Criteria	Frequency	Acceptance Criteria	Frequency	
Dissolved inorganic nutrients	Technicon II Autoanalyzer	4-5	r ≥.999	Prior to analytical run	PD from initial ≤15%;	Every 20 samples	Investigate, recalibrate
Dissolved organic carbon	Shimadzu 5000 Carbon Autoanalyzer	4-5	r ≥ .995	Prior to analytical run	PD from initial ≤15%;	Every 20 samples	Investigate, recalibrate
Total dissolved nitrogen and phosphorus	Technicon Autoanalyzer II	4-5	RSD = 85%-115%	Prior to analytical run	PD from true value ≤15%;	Every 20 samples	Investigate, recalibrate
Particulate carbon and nitrogen	Exeter Analytical Elemental Analyzer Model CE 440	1	NA	Prior to analytical run	PD from initial ≤ 15%	Every 20 samples	Investigate, recalibrate
Particulate phosphorus	Technicon II Autoanalyzer	4-5	r≥0.995	Prior to analytical run	PD from initial ≤ 15%	Every 20 samples	Investigate, recalibrate
Biogenic silica	Technicon II Autoanalyzer	4-5	r≥0.995	Prior to analytical run	PD from Initial ≤ 15%	Every 20 samples	Investigate, recalibrate
Urea	Shimadzu UV_Vis Spectrophotometer	5	$r^2 \geq 0.995$	Prior to analysis	<10% RPD from initial	Every 20 samples	Investigate, recalibrate
Chlorophyll *a* and phaeophytin	Model 10AU Turner Designs Fluorometer	6	r ≥ 0.995	Prior to analytical run	PD from initial ≤10%	Twice daily	Investigate, recalibrate
Total Suspended Solids (TSS)	Mettler 5- Place Balance	NA	Professionally Calibrated to Agree with NIST traceable Calibration Weights	Annually	PD less than 1% from reference weights	Daily	Professional Service requested for PD over 5%
Dissolved oxygen and respiration	Radiometer Titralab ™	NA	NA	NA	NA	NA	Investigate, recalibrate
Respiration	Radiometer Titralab ™	1	NA	Prior to Analysis for each survey	NA	NA	Investigate, recalibrate
Primary Production by ^{14}C	Beckman Model LS 3801 scintillation counter						Investigate, recalibrate

15.0 DOCUMENTATION, DATA REDUCTION, AND REPORTING

15.1 Data Recording

All data will be initially recorded either (1) electronically onto computer storage media from BOSS or other laboratory systems or (2) manually into bound laboratory notebooks or onto established data forms. All notes will be written in black ink. Corrections to hand-entered data will be initialed, dated, and justified. Corrections to electronically captured data (*e.g.*, electronic "spikes") will be documented on a hard-copy plot of the data. Completed data forms or other types of hand-entered data will be signed and dated by the individual entering the data. Direct-entry and electronic data entries will indicate the person collecting or entering the data. It will be the responsibility of the laboratory managers to ensure that all data entries and hand calculations are verified in accordance with procedures described in Section 16 (below). In addition to these documentation procedures, station logs associated with field and laboratory custody and tracking will be kept in survey notebook for each survey. These notebooks will be stored in the physical oceanography laboratory under the supervision of Mr. Wayne Trulli.

15.2 Data Reduction

15.2.1 Hydrographic and Navigation Data

The hydrographic data generated during the survey will consist of rapidly sampled, high-resolution measurements of conductivity, temperature, depth, DO, transmissometry, underwater light levels, total incident radiation, altitude above bottom, and bathymetry. The BOSS data-acquisition software assigns an unique data filename to each vertical profile made during the survey. All data will be electronically logged with date, time, and concurrent GPS/LORAN vessel-position data. Battelle's NAVSAM™ software will be used to convert the raw engineering data into concentration units using factory or laboratory calibration coefficients. The irradiance data from the light sensor table will be electronically and manually reviewed for proper operation of the two light sensors. Shading will be noted in the station log and the data qualified accordingly. The converted hydrocast will be plotted in high resolution, parameter versus depth graphic form for visual inspection of data representativeness. After editing is complete, the hydrocast data that corresponds to the discrete water samples will be combined with the wet chemistry to develop calibrated dissolved oxygen and chlorophyll *a* profile data. NAVSAM™ will create a Microsoft Access database file consisting of two tables. One table will contain the downcast data that will exclude the ship upward motions and be averaged to 0.5-m depth bins. A second table includes the upcast data corresponding to the average of the data within +/- 5 seconds in the closing of the rosette bottle at each sampling depth. The database file will serve as an export file to the EM&MS database. The data reductions are described by Weiss (1970) and Fofonoff and Millard (1983).

15.2.2 Subcontractor Laboratory Data

All data generated by Battelle's subcontractors will be either electronically transferred from the instrument or manually read from the instrument display (or optical field of a microscope) and entered into a loading application, provided by the Battelle Data Management team. Data in laboratory notebooks will be manually entered into the loading application. All data reduction will be performed electronically either by the instrument software or in a spreadsheet and will be validated according to procedures described in Section 16. The format for final data submission is described below.

15.3 Reporting Data to be Loaded into the Database

All field and laboratory data to be loaded into the EM&MS will be submitted to Battelle in electronic format. The field data will be available for data loading directly off the ship. The laboratories will be supplied a loading application that will increase data quality and efficiency. These applications eliminate the need for data reporting formats and deliver many of the quality control checks upstream to the laboratories.

15.3.1 Navigation and Sample Collection Data

Navigation and sample collection data will be processed on-board the survey vessel and be ready for loading into EM&MS upon arrival at Battelle. A database application developed as part of the NAVSAM system will query the on-board database tables for the fields necessary to populate the *Event, Station, Sample* and *Bottle* tables. The data will be loaded into the EM&MS database by clicking a button. All database constraints developed by MWRA will be applied to the tables so that the data are checked during the insert.

15.3.2 Hydrographic Data

Battelle will also load into the database the following two types of data collected with the BOSS sensor package:

- Date, time, location, and corrected sensor data associated with each water sample (upcast data)
- Date, time, location, and corrected vertical profile sensor data that has been bin-averaged into 0.5-m bins (downcast data)

A database application will be used to load the hydrographic data from the processing database directly into the EM&MS database. Table 16 shows the database codes for the hydrographic parameters. Database constraints will be in place to provide an initial check of the data integrity and validity.

Table 17. Database Codes for Hydrographic Parameters

Parameter	Param_Code	Unit_Code	Instr_Code	Meth_Code
Conductivity	CONDTVY	mmhos/cm	CTD1	boss
Dissolved Oxygen	DISS_OXYGEN	mg/L	DO1	boss
Fluorescence	FLUORESCENCE	ug/L	FLU1	boss
in situ Irradiance level	LIGHT	uEm-2sec-1	LIG2	boss
Water Pressure	PRESSURE	db	CTD1	boss
Salinity	SAL	PSU	CTD1	boss
Density as measured by Sigma_t	SIGMA_T		CTD1	boss
Surface irradiance level	SURFACE_IRRAD	uEm-2sec-1	LIG2	boss
Temperature	TEMP	C	CTD1	boss
Transmissivity	TRANS	m-1	T1R25	boss

15.3.3 Analytical and Experimental Data

The data reporting for analytical and experimental data begins with the Battelle Data Management Team who populate a loading application that is then sent to the laboratory for their data entry. As defined above, the collection data from field activities are delivered to the data manager as an Access database. Sample Ids and analysis protocols are extracted from this database and used to populate a database within the loading application. A separate loading application is prepared for each data deliverable. Data contributors open the database and are presented with a form that already contains the Sample Ids and analyte list for their data submittal (Figure 14). The laboratory enters the results and other supporting information such as qualifiers. All entries are constrained by the rules of EM&MS. Errors are caught on entry and fixed by the data contributor. Primary keys are in-place so duplication can not occur. Entry applications are developed on an individual laboratory basis. Laboratory staff receives one day of training on the application prior to their first set of samples. When data entry is complete, the database is sent back to Battelle. Laboratories with existing data processing capability will be supplied a loading application that can import their final spreadsheet and then run the quality control checks. The laboratory will have to meet their own internal laboratory format for the data to load successfully.
The loading application gives the laboratory several function buttons (Figure 15). These include hardcopy report, quality control checks, exception report, and analysis summary. The hardcopy report allows the laboratory to create a hardcopy report to check for entry errors and to submit a final report to Battelle with the data deliverable. The quality control checks are comprised of the applicable sections of EM&MS check and constraints scripts and also perform checks for outliers. This report gives the data

contributor a chance to confirm the reasonableness of their data prior to submission to Battelle. The exception report checks the data that was expected against the results loaded. The data contributor must account for any entries in the exception report. The analysis report produces a report of the number of analyses by analyte. A copy of this report is included with the data deliverable and with the invoice for the analyses. Within the loading application, the data entered by the laboratory is translated into the correct codes and inserted into database tables with the same structure as the matching EM&MS table. Table 17 shows the qualifiers to be used by the laboratory. Database codes for plankton taxonomy are presented in Table 18. Table 19 shows the analytical parameters, codes, and units of measure for the analytes collected under this task. The database codes are described in Table 20. The laboratory will have the ability to add additional codes to describe their results but the new qualifiers will be highlighted in the exception report. Battelle will notify MWRA concerning the new qualifier and will adjust the code table in the application to agree with any changes to the EM&MS code list table. MWRA has the responsibility for maintaining the code list for the EM&MS.

Table 18. Laboratory Qualifiers

Qualifier	Description	Value Reported?
	Value is not qualified	yes
a	Not detected and <MDL	no
A	Over maximum detection limit (TNTC)	yes
B5	Blank-corrected, blank <5 x MDL	yes
b	Below limit of quantitation	yes
c	Ambient	yes
e	Not reported	no
E	Calibration level exceeded	yes
f	Value reported <MDL	yes
g	Recovery outside DQO	yes
G	Coalluting compound interferences	yes
h	Below standard curve	yes
I	Interference from standard	yes
j	Estimated value above detection limit	yes
l	Dark bottle	yes
le	Dark, not reported	yes
L	Analytical concentration reported from dilution	yes
m	Initial	yes
n	Light bottle	yes
PRO	Abundance recorded for a fraction or portion of the sample collected	yes
p	Lab sample bottles mislabeled - caution data use	yes
r	Precision >DQO	yes
s	Suspect	yes
T	Holding time exceeded	yes
v	Arithmetic mean	yes
x	Matrix interference	yes
1	Present	yes
2	Absent	yes
P	Present, uncounted	yes

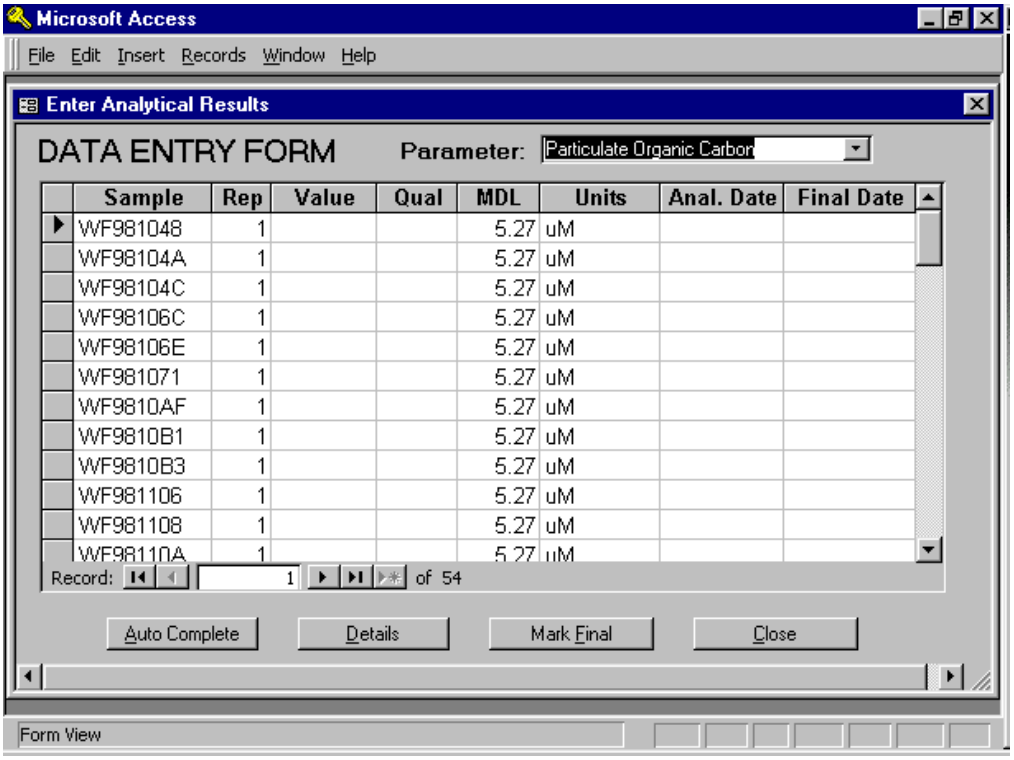


Figure 14. Example of Loading Application Data Entry Form

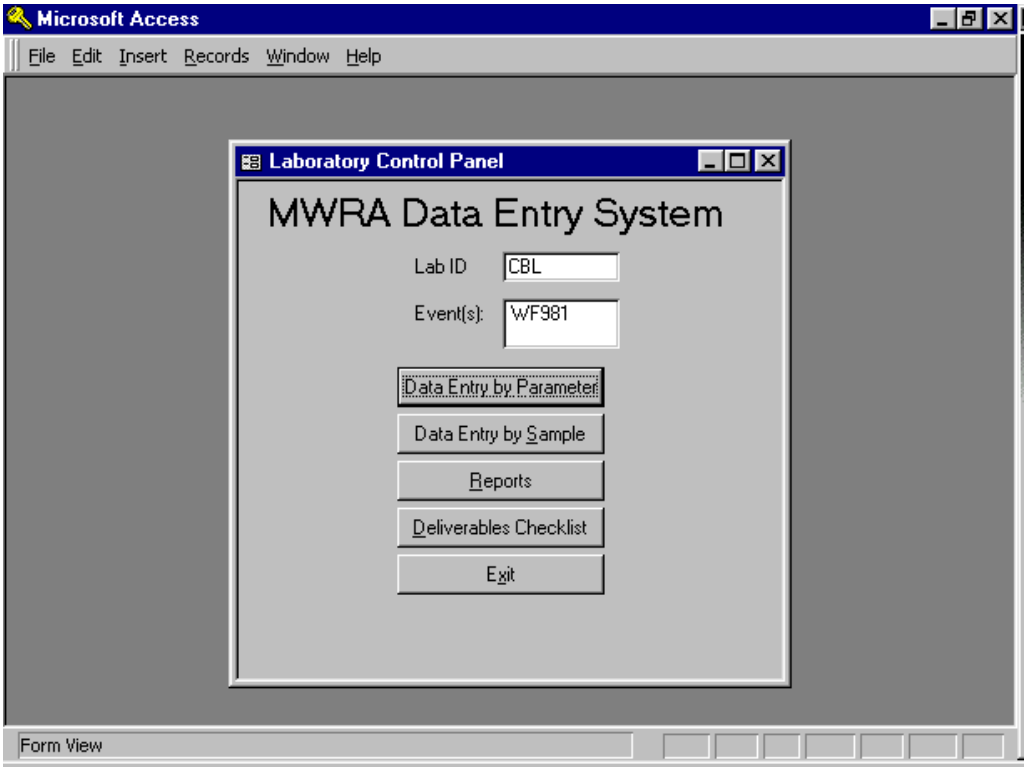


Figure 15. Loading Application Main Menu

Table 19. Database Codes for Plankton Taxonomy

Plankton Analysis	Unit_Code	Meth_Code	Biomass_Unit_Code	Anal_Lab_ID
Whole-Water Phytoplankton	E6CELLS/L	COU_WW	ug/L	UMD
Screened Phytoplankton	CELLS/L	SCR20U	ug/L	UMD
Zooplankton	ind/m3	COU_ZO	ug/L	UMD

Table 20. Database Codes for Chemistry Analytical and Experimental Parameters

Parameter	Param_Code	Unit_Code	Anal_Lab_ID	Instr_Code	Meth_Code
Chlorophyll a	CHLA	ug/L	BOS	FLU5	ARAR92
Dissolved Oxygen	DISS_OXYGEN	mg/L	BOS	RTL	OUD88
Respiration	Respiration	uM/hr	BOS	RTL	SP1972
Total Suspended Solids (TSS)	TSS	mg/L	BOS	METLR	SOP5-053
Phaeophytin	MWRA79	ug/L	BOS	FLU5	ARAR92
Biogenic Silica	BIOSI	uM	CBL	T2A	PAA73
Dissolved Organic Carbon	DOC	uM	CBL	SHMDZ	SUZ92
Particulate Organic Carbon	POC	uM	CBL	EA-CE440	440.0
Particulate Organic Nitrogen	PON	uM	CBL	EA-CE440	440.0
Total Dissolved Nitrogen	TDN	uM	CBL	T2A	VALD81
Total Dissolved Phosphorus	TDP	uM	CBL	T2A	VALD81
Urea	57-13-6	uM	MBL	SPECPH	RB80_PH87
Ammonium	NH4	uM	URI	T2A	SOL69
Nitrite	NO2	uM	URI	T2A	BR52_MR63
Nitrate	NO3	uM	URI	T2A	BR52_MR63
Particulate Phosphorus	PARTP	uM	URI	T2A	SOL80
Phosphate	PO4	uM	URI	T2A	MURPH62
Silicate	SIO4	uM	URI	T2A	BREW66
Areal Production	AREAL_PROD	mgCm-2d-1	URI	L3801	ALBRO98
Daily Production	DAILY_PROD	mgCm-3d-1	URI	L3801	ALBRO98
Potential Productivity	Pmax	mgCm-3h-1	URI	L3801	ALBRO98
Alpha parameter for productivity curve	Alpha	ALPHA	URI	L3801	ALBRO98
Beta parameter for productivity curve	Beta	ALPHA	URI	L3801	ALBRO98
Hourly Production	HOURLY_PROD	mgCm-3h-1	URI	L3801	ALBRO98
Incubation Time	MWRA63	hours	URI	NA	NA
Temperature	TEMP	C	URI	NA	NA
Light Exposure	MWRA53	uEm-2sec-1	URI	NA	NA

NA: Not applicable

Table 21. Description of Database Codes

Field_Name	Code	Description
ANAL_LAB_ID	BOS	Battelle Ocean Sciences, Duxbury, MA
ANAL_LAB_ID	CBL	Chesapeake Biological Laboratory, Solomons, MD
ANAL_LAB_ID	MBL	Marine Biological Laboratory, Woods Hole, MA
ANAL_LAB_ID	UMD	University of Massachusetts, Dartmouth, MA
ANAL_LAB_ID	URI	University of Rhode Island, Narragansett, RI
INSTR_CODE	ALPAA	Alpchem Autoanalyzer
INSTR_CODE	CTD1	Seabird 9 CTD
INSTR_CODE	DO1	Seabird/Beckman DO Probe
INSTR_CODE	EA-CE440	Exeter Analyzer Model CE-440
INSTR_CODE	FLU1	Chelsea Fluorometer
INSTR_CODE	FLU5	Turner Designs Model 10 Fluorometer
INSTR_CODE	L3801	Beckman Liquid Scintillation Counter Model 3801
INSTR_CODE	LIG2	Biospherical Irradiance Sensor
INSTR_CODE	METLR	Mettler balance
INSTR_CODE	PE5100	Perkin-Elmer Model 5100 AA Graphite Furnace
INSTR_CODE	RTL	Radiometer Autotitrator
INSTR_CODE	SHMDZ	Shimadzu 5000 Carbon Analyzer
INSTR_CODE	T1R25	Sea Tech 25 cm Transmissometer
INSTR_CODE	T2A	Technicon Autoanalyzer II
METH_CODE	200.2	EPA Method 200.2 and EPA (1993)
METH_CODE	440.0	EPA Method 440.0 (March 1997)
METH_CODE	ALB93	Albro *et al.* (1993)
METH_CODE	ARAR92	Arar and Collins (1992)
METH_CODE	BOSS	Battelle Ocean Sampling System
METH_CODE	BR52_MR63	Bendschneider and Robinson (1952); Morris and Riley (1963)
METH_CODE	BREW66	Brewer and Reilly (1966)
METH_CODE	COU_WW	Enumeration method for whole-water phytoplankton (Hunt *et al*. 1998)
METH_CODE	COU_ZO	Enumeration method for zooplankton (Hunt *et al*.. 1998)
METH_CODE	ALBRO98	Productivity calculaated as in Albro *et al*. 1998 Water Column CW/QAPP
METH_CODE	MURPH62	Murphy and Riley (1962)
METH_CODE	OUD88	Oudat *et al.* (1988)
METH_CODE	PAA73	Paasche (1973)
METH_CODE	RB80_PH87	Price and Harrison (1987)
METH_CODE	SCR20U	Large dinoflag. screening technique 20 microns
METH_CODE	SOL69	Solorzano (1969)
METH_CODE	SOL80	Solorzano and Sharp (1980)
METH_CODE	SOP5-053	Battelle Ocean Sciences SOP No. 5-053
METH_CODE	SP1972	Strickland and Parsons (1972)
METH_CODE	SUZ92	Suzuki *et al*. (1992)
METH_CODE	VALD81	Valderrama (1981)
UNIT_CODE	ALPHA	mgCm-3h-1uE-1m2s
UNIT_CODE	C	Degrees Celsius
UNIT_CODE	CELLS/L	Cells per liter
UNIT_CODE	db	Decibar
UNIT_CODE	E6CELLS/L	Millions of cells per liter
UNIT_CODE	hours	Hours
UNIT_CODE	ind/m3	Individuals per cubic meter
UNIT_CODE	m-1	Inverse meters
UNIT_CODE	mg/L	Milligrams per liter
UNIT_CODE	mgCm-2d-1	Milligrams of carbon per square meter per day
UNIT_CODE	mgCm-3d-1	Milligrams of carbon per cubic meter per day
UNIT_CODE	mgCm-3h-1	Milligrams of carbon per cubic meter per hour
UNIT_CODE	mmhos/cm	Millimhos per centimeter
UNIT_CODE	PSU	Practical salinity units
UNIT_CODE	uEm-2sec-1	Micro-Einsteins per square meter per second
UNIT_CODE	ug/L	Micrograms per liter
UNIT_CODE	uM	Micromoles per liter
UNIT_CODE	uM/hr	Micromoles per liter per hour

15.4 Loading Analytical and Experimental Data into the Harbor Studies Database

Data submissions from the laboratory are the final loading applications. The submissions are logged in upon receipt and a copy is maintained on file under the login id. Data are loaded into a temporary table space by a button on the application. A transfer script will copy the data into the proper table in Battelle's copy of the EM&MS. Data from the laboratories receive a quality assurance review after the data has been synthesized into a data report. Any issues are corrected in the database and the well-documented script is supplied to MWRA with the export of the database. The MWRA check script will be run on the database as a batch job each night. Any issues will be sent to the data manager and MWRA via email. Any changes to the database as a result of quality control checks will also be submitted to MWRA with the data export.

15.5 Reporting Data to MWRA

The data contained in each hard copy data report are submitted to MWRA as a database export. The supporting documentation files are included with the data submission. Data deliverables will be combined only with permission from MWRA.

16.0 DATA VALIDATION

The data validation procedures for this project are defined in the HOM3 Quality Management Plan. As a part of data validation, each Task Leader ensures that:

- Any data that are hand-entered (*i.e.*, typed) are validated by qualified personnel prior to use in calculations or entry into the database.

- All manual calculations are performed by a second staff member to verify that calculations are accurate and appropriate.

- Calculations performed by software are verified at a frequency sufficient to ensure that the formulas are correct, appropriate, and consistent, and that calculations are accurately reported. All modifications to data reduction algorithms are verified prior to submission of data to the Authority.

- Electronic data loading and transfer are swift and routine; data fields and formats are defined in the CW/QAPPs. Electronic submissions are loaded to temporary files prior to incorporation into the database, and are analyzed selectively using methods such as scatter plots, univariate and multivariate analyses, and range checks to identify suspect values. Routine system back-ups are performed daily.

- Once data have been generated and compiled in the laboratory, senior project scientists review data to identify and make professional judgments about any suspicious values. All suspect data are reported with a qualifier. This data may not be used in calculations or data summaries without the review and approval of a knowledgeable Senior Scientist. No data measurements are eliminated from the reported data or database and data gaps are never filled based on other existing data. If samples are lost during shipment or analysis, it is documented in the data reports to the Authority and noted in the database.

17.0 PERFORMANCE AND SYSTEM AUDITS

The Battelle QA Officer for the Harbor and Outfall Monitoring Project is Ms. Rosanna Buhl. She will direct the conduct of at least one systems audit to ensure that Tasks 9-15 are carried out in accordance with this CW/QAPP. A systems audit will verify the implementation of the Quality Management Plan and this CW/QAPP for the work conducted in the Water Quality monitoring.

Tabular data reported in deliverables, and associated raw data generated by Battelle will be audited under the direction of the Project QA Officer. Raw data will be reviewed for completeness and proper documentation. For electronically acquired data (*e.g.*, navigational data), Ms. Buhl will verify that computer software used to process the data has been validated. Errors noted in data audits will be communicated to analysts and corrected data will be verified.

Audits of the data collection procedures at subcontractor laboratories will be the responsibility of the Subcontractor. Each subcontractor is fully responsible for the QA of the data it submits. Data must be submitted in CW/QAPP-prescribed formats; no other will be acceptable. During the time work is in progress, an inspection will be conducted by the subcontractor QA Officer or their designee to evaluate the laboratory data-production process. All data must be reviewed by the subcontractor QA Officer prior to submission to the Battelle Database Manager and must be accompanied by a signed QA statement that describes the types of audits and reviews conducted and any outstanding issues that could affect data quality and a QC narrative of activities.

The Battelle QA Officer will conduct an initiation audit and, as needed, a laboratory inspection to access compliance with the Quality Management Plan and this CW/QAPP. Performance audits, procedures used to determine quantitatively the accuracy of the total measurement system or its components, will be the responsibility of the subcontractor laboratory and may include internal performance evaluation samples and participation in external certification programs.

18.0 CORRECTIVE ACTION

All technical personnel share responsibility for identifying and resolving problems encountered in the routine performance of their duties. Dr. Carlton Hunt, Battelle's Project Manager, will be accountable to MWRA and to Battelle management for overall conduct of the Harbor and Outfall Monitoring Project, including the schedule, costs, and technical performance. He is responsible for identifying and resolving problems that (1) have not been addressed timely or successfully at a lower level, (2) influence multiple components of the project, (3) necessitate changes in this CW/QAPP, or (4) require consultation with Battelle management or with MWRA.

Identification of problems and corrective action at the laboratory level (such as meeting data quality requirements) will be resolved by laboratory staff or by Subcontractor Managers (see Figure 5). Issues that affect schedule, cost, or performance pf the water-column monitoring tasks will be reported to the Task Leader or to the Battelle Project Manager. They will be responsible for evaluating the overall impact of the problem on the project and for discussing corrective actions with the MWRA Project Manager. Problems identified by the QA Officer will be reported and corrected as described in Section 17.0.

19.0 REPORTS

Water column surveys (Tasks 9 and 10), *in situ* data processing (Task 6), data loading and quality assurance (Tasks 5 and 7), results from remote sensing (Tasks 12 and 13), and sample analysis (Tasks 14 and 15) will be reported in survey reports (Tasks 9 and 10) and data reports (Tasks 14 and 15). Data synthesis will be reported under Task 33.

Survey-related deliverables that will be generated under this CW/QAPP include:

- 51 Survey Plans (one for each of the Nearfield water column surveys; farfield surveys plans will be combined with the Nearfield Plans)
- 51 Survey Reports (one for each of the Nearfield water column surveys; farfield surveys reports will be combined with the Nearfield Reports)
- 51 Rapid Phytoplankton Email Reports
- 51 Email Survey Summaries including any violations of Contingency Plan thresholds
- 15 Nutrient Data and Respiration/Productivity Data Reports
- 15 Phytoplankton Data and Zooplankton Data Reports

19.1 Survey-Related Reports

For each nearfield survey, one survey plan, one survey email, and one survey report will be prepared. For combined nearfield and farfield surveys, these documents will also be combined. A total of 51 nearfield and combined nearfield/farfield surveys will be reported as described below.

19.1.1 Survey Plans

Survey plans will be prepared for each survey conducted. In the case of combined surveys, a single plan covering all aspects the combined surveys will be submitted to MWRA. Each survey plan will follow the guidelines established by U.S. Environmental Protection Agency for use of the OSV *Anderson* and will be submitted as a final unbound, double side copy on 3-hole paper at least one week prior to the start of the survey. Each survey plans will include the following information:

- Purpose, background, and data use for survey
- Schedule of operations
- Specific location and coordinates of each station
- Survey/sampling methods
- Navigation and positioning control
- Vessel, equipment, and supplies
- Scientific party
- Documentation of any deviations from this CW/QAPP
- Tide and tidal current data for each survey day (determined 0.2 nm south of Boston Light using Micronautics, Inc. Tide.1 and Tide.2 software)

19.1.2 Survey Email Summary

A survey summary will be delivered survey submitted to MWRA via Email within 1 week of completion of each survey. This Email will include a summary of the survey operational dates, weather conditions, stations not sampled and reason, summary of preliminary water quality observations, any threshold values

exceeded, deviations from survey scope, results of the rapid phytoplankton analysis, observations of marine mammal sitings, and identify technical problems encountered and resolutions.

19.1.3 Survey Reports

Survey reports will describe the survey conducted, stations occupied, measurements made, samples collected, problems experienced, and general observations of water quality including any violations of Contingency Plan caution or warning thresholds, and summarize observations made by the certified whale observer. Survey reports are expected to be 4-5 pages of text with accompanying station maps and sample collection table. The sample collection table will be a tabular summary of stations occupied, station locations, and samples collected. This data will be generated directly from the Battelle HOM3 database. Any deviations from this CW/QAPP, not known at the time of survey plan preparation, will also be incorporated into the survey reports. Two unbound, single-sided copies of the draft survey report will be submitted to MWRA no later than two weeks after the completion of each survey. MWRA's comments on the report will be due to Battelle two weeks after receipt of the draft report. The final survey report, addressing MWRA's comments, will be due to MWRA two weeks after receipt of the comments. If MWRA does not submit comments within the two-week period, the draft survey report will be considered final.

19.2 Data Reports

Five Nutrient and five Respiration/Productivity data reports will be submitted to MWRA each per year. Each report is final. The data reports are formatted to provide a user-friendly view of the data. The data reports are created directly from the Battelle version of the EM&MS.

The format and the content of the data report are reviewed with the MWRA technical task leader prior to the submission of the first set. All subsequent reports are submitted in this format.

19.2.1 Nutrient Data Reports

The five Nutrient Data Reports due each year will contain tabular summaries of concentrations of all nutrient species measured, chlorophyll *a*, DO, and TSS for each bottle sampled and analyzed. The report will also include hydrographic data (salinity, temperature, DO, chlorophyll fluorescence, optical beam transmittance, light radiance, and sensor altitude above the seafloor), Secchi disk depth, plume tracking in situ sensor data, and meteorological data from Deer Island.

19.2.2 Respiration/Productivity Data Reports

The five Respiration/Production Data Reports due each year will include a tabular summaries of water-column respiration rates and primary production calculations including the P_{max}, and P(I) analyses will be provided for each sample depth or profile measured. Electronic exports of these data will be provided to MWRA 30 days after the data report is delivered.

19.2.3 Plankton Data Reports

Five plankton data reports will be prepared each year and submitted to MWRA. These data reports will contain tabular summaries of phytoplankton and zooplankton counts and identifications. Electronic exports of these data will be provided to MWRA 30 days after the data report is delivered. In addition to these data reports, a Sensor Data Processing Letter report will be submitted monthly. The report letter

will summarize sensor processing completed each month including any problems encountered and a list of data provided to the database administrator.

19.3 Synthesis Reports

The data delivered above will be used in synthesis reports prepared under Task 33.2 (Periodic Water Column Reports), Task 33.3 (Annual Water Column Reports), and Task 33.10 (Nutrient Issues Reviews). A detailed outline of each of the above synthesis reports will be prepared for MWRA approval. Following approval, a draft report will be prepared and submitted to MWRA. MWRA comments on each report will be provided to Battelle within 2 weeks of report receipt. Final reports, addressing MWRA comments, will be due to MWRA within two weeks of comment receipt.

19.3.1 Periodic Water Column Reports

These twice annual periodic water column reports provide rapid chronicling the basic results from Task 6, and Tasks 9 through 15. The report will present meteorological, oceanographic, chemical, and biological conditions over a 6 month period. The semi-annual water quality reports provide quick-look summaries of patterns in the water column data, highlight unusual events of the period, and relate the information to the caution and warning thresholds of the Outfall Monitoring Plan. Any exceedances of these thresholds will be summarized in this report.

The Periodic Water column Reports will subscribe to the outline shown below. Standardized graphic presentations supporting the discussions will be included:

- Executive Summary (including summary of any thresholds triggered)
- Introduction (program overview, report purpose, report organization)
- Methods (References the water column CW/QAPP, describes methodological changes, and scope deviations).
- Data Summary (Chronological tabularization of summary data for each water column survey performed during the reporting period. Data from Tasks 12, 13, 26 and 28 will be summarized in this section.)
- Water Column Results. (Includes a brief discussion of the water column results and characteristics to introduce the section)
- Physical characteristics (standardized graphics of nutrient, chlorophyll *a*, and DO by survey)
- Nutrients
- Chlorophyll *a*
- Dissolved oxygen.
- Results Summary (brief discussion of primary productivity, respiration, and plankton data).
- Primary Production (Description of the spatial and temporal characteristics of areal, chlorophyll specific and potential primary production)
- Respiration (description of water column respiration)
- Phytoplankton (Seasonal trends in abundance, nearfield community structure, regional assemblages, nuisance algae)
- Zooplankton (Seasonal trends in abundance, nearfield community structure, regional assemblages)
- Major events (Summary of any major spatial-, temporal-, or regional-scale events, major deviations from the baseline conditions, and summary violations of the caution and warning thresholds).

- References (list of all references cited)
- Appendices (Additional graphics from the various surveys, including individual station profile plots, photosynthesis-irradiance curves, and other routine figures and graphs that convey the basic results of the measurements made on the water column surveys. Format after content of Murray *et al.* (1998)

19.3.2 Annual Water Column Report

The annual water quality report will synthesize results from water column monitoring activities for each calendar year. For the last year of the baseline monitoring (1998), it will describe the status of the ecosystem, including annual and seasonal patterns. The annual report will provide statistical descriptions of critical parameters and evaluate critical interactions among biological, physical, and chemical factors. The report also will include summaries of annual minimums and maximums (identified according to time and location), frequency distributions, seasonal, and annual averages as appropriate to the monitoring caution and threshold values. The annual report for 1998 will seek to summarize the year in the context of the previous baseline years and the variability observed throughout the baseline periods in preparation for the post-discharge monitoring.

After the commissioning of the Massachusetts Bay effluent outfall, the annual report will focus on assessing the status of the ecosystem in comparison to baseline monitoring results and the caution and warning thresholds. Statistical comparisons and tests for detectable change will be conducted. Should any violations of the relevant monitoring thresholds be observed, Battelle will assess the likely cause and determine whether the cause can be attributed to the outfall.

Each annual water column report will address the following areas:

- Executive Summary (including summary of any thresholds triggered and possible factors responsible)
- Introduction (Program Overview, report purpose, report organization)
- Data Sources and Overview of each Years' Program
- Environmental Setting: Physical Oceanography and Meteorology (Temperature cycle, Salinity, Water Column Stratification, Water Mass Source and Movement, Rainfall, Light cycle)
- Nutrients (Annual cycle in the Nearfield and in Massachusetts Bay and Cape Cod Bay)
- Chlorophyll (Nearfield. Regional and Inter-annual Comparisons)
- Dissolved Oxygen (Annual cycle in the Nearfield and in Massachusetts Bay and Cape Cod Bay, seasonal decline in bottom waters of the Nearfield and Stellwagen basis)
- Productivity and Respiration (Seasonal and Annual Production, chlorophyll specific measures of production, Water column respiration)
- Plankton (Abundance and seasonal succession, regional comparisons, Inter-annual comparisons, and algal nuisance species
- Overview of Annual Results (Integration and Synthesis)
- References

19.3.3 Nutrient Issues Review

This report draws from a variety of reports and data to evaluate the potential for response related to relocation of the MWRA outfall and associated nutrients in Massachusetts and Cape Cod Bays. Topics may vary as information and data gaps are identified. Therefore the final content must be defined and approved prior to starting report preparation. Tentatively the first nutrient review developed under this

CW/QAPP will focus on evaluating the pre-discharge water quality information (through 1998) and the responses in Massachusetts Bay through 1999. The report will focus on describing and summarizing expected impacts from the relocated discharge. The 1998 report may focus on the trophic status of Massachusetts and Cape Cod Bays, including gradients and dilution of the present Deer Island effluent, nutrient processes in the column and benthos, and budgets for the system. The report may focus heavily on any predicted responses and impact from relocated outfall and those indicators that will provide the most effective for detecting change. The 1999 report may be used to evaluate any effects from outfall relocation on nutrient related process in Boston Harbor and Massachusetts Bay.

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Appendix A

Nearfield and Farfield Sampling Plans

Table A-1. Nearfield Water Column Sampling Plan (3 Pages). Dissolved Oxygen Quality Assurance Samples will be Collected at the First and Last DO Calibration Station of Each Day.

Nearfield Water Column Sampling Plan

StationID	Depth (m)	Station Type	Depths	Total Volume at Depth (L)	Number of 9-L GoFlos	Dissolved Inorganic Nutrients	Dissolved Organic Carbon	Total Dissolved Nitrogen and	Particulate Organic Carbon and	Particulate	Biogenic silica	Chlorophyll a	Total Suspended Solids	Dissolved Oxygen	Rapid Analysis Phytoplankton	Whole Water Phytoplankton	Screened Water Phytoplankton	Zooplankton	Urea	Respiration	Photosynthesis by carbon-14	Dissolved Inorganic Carbon
			Protocol Code			IN	OC	NP	PC	PP	BS	CH	TS	DO	RP	WW	SW	ZO	UR	RE	AP	IC
			Volume (L)			1	0.1	0.1	1	0.6	0.3	0.5	1	1	4	1	4	1	0.1	1	1	1
			1_Bottom	8.5	2	1	1	1	2	2	2	1	2	1								
			2_Mid-Bottom	2.5	1	1						1		1								
N01	30	A	3_Mid-Depth	10	2	2	1	1	2	2	2	2	2	1								
			4_Mid-Surface	2.5	1	1						1		1								
			5_Surface	8.5	2	1	1	1	2	2	2	1	2	1								
			1_Bottom	1	1	1																
			2_Mid-Bottom	1	1	1																
N02	40	E	3_Mid-Depth	1	1	1																
			4_Mid-Surface	1	1	1																
			5_Surface	1	1	1																
			1_Bottom	1	1	1																
			2_Mid-Bottom	1	1	1																
N03	44	E	3_Mid-Depth	1	1	1																
			4_Mid-Surface	1	1	1																
			5_Surface	1	1	1																
			1_Bottom	15.5	2	1	1	1	2	2	2	1	2							6	1	1
			2_Mid-Bottom	4.5	1	1						1		1							1	1
N04	50	D+	3_Mid-Depth	22.1	2	2	1	1	2	2	2	2	2			1	1		1	6	1	1
		R+	4_Mid-Surface	4.5	1	1						1		1							1	1
		P	5_Surface	20.6	2	1	1	1	2	2	2	1	2			1	1		1	6	1	1
			6_Net Tow															1				
			1_Bottom	1	1	1																
			2_Mid-Bottom	1	1	1																
N05	55	E	3_Mid-Depth	1	1	1																
			4_Mid-Surface	1	1	1																
			5_Surface	1	1	1																
			1_Bottom	1	1	1																
			2_Mid-Bottom	1	1	1																
N06	52	E	3_Mid-Depth	1	1	1																
			4_Mid-Surface	1	1	1																
			5_Surface	1	1	1																
			1_Bottom	10.5	2	1	1	1	2	2	2	1	2	3								
			2_Mid-Bottom	2.5	1	1						1		1								
N07	52	A	3_Mid-Depth	10	2	2	1	1	2	2	2	2	2	1								
			4_Mid-Surface	2.5	1	1						1		1								
			5_Surface	10.5	2	1	1	1	2	2	2	1	2	3								
			1_Bottom	1	1	1																
			2_Mid-Bottom	1	1	1																

Nearfield Water Column Sampling Plan

StationID	Depth (m)	Station Type	Depths	Total Volume at Depth (L)	Number of 9-L GoFlos	Dissolved Inorganic Nutrients	Dissolved Organic Carbon	Total Dissolved Nitrogen and	Particulate Organic Carbon and	Particulate	Biogenic silica	Chlorophyll a	Total Suspended Solids	Dissolved Oxygen	Rapid Analysis Phytoplankton	Whole Water Phytoplankton	Screened Water Phytoplankton	Zooplankton	Urea	Respiration	Photosynthesis by carbon-14	Dissolved Inorganic Carbon
			Protocol Code			IN	OC	NP	PC	PP	BS	CH	TS	DO	RP	WW	SW	ZO	UR	RE	AP	IC
N08	35	E	3_Mid-Depth	1	1	1																
			4_Mid-Surface	1	1	1																
			5_Surface	1	1	1																
			1_Bottom	1	1	1																
			2_Mid-Bottom	1	1	1																
N09	32	E	3_Mid-Depth	1	1	1																
			4_Mid-Surface	1	1	1																
			5_Surface	1	1	1																
			1_Bottom	8.5	2	1	1	1	2	2	2	1	2	1								
			2_Mid-Bottom	2.5	1	1						1		1								
N10	25	A	3_Mid-Depth	10	2	2	1	1	2	2	2	2	2	1								
			4_Mid-Surface	2.5	1	1						1		1								
			5_Surface	8.5	2	1	1	1	2	2	2	1	2	1								
			1_Bottom	1	1	1																
			2_Mid-Bottom	1	1	1																
N11	32	E	3_Mid-Depth	1	1	1																
			4_Mid-Surface	1	1	1																
			5_Surface	1	1	1																
			1_Bottom	1	1	1																
			2_Mid-Bottom	1	1	1																
N12	26	E	3_Mid-Depth	1	1	1																
			4_Mid-Surface	1	1	1																
			5_Surface	1	1	1																
			1_Bottom	1	1	1																
			2_Mid-Bottom	1	1	1																
N13	32	E	3_Mid-Depth	1	1	1																
			4_Mid-Surface	1	1	1																
			5_Surface	1	1	1																
			1_Bottom	1	1	1																
			2_Mid-Bottom	1	1	1																
N14	34	E	3_Mid-Depth	1	1	1																
			4_Mid-Surface	1	1	1																
			5_Surface	1	1	1																
			1_Bottom	1	1	1																
			2_Mid-Bottom	1	1	1																
N15	42	E	3_Mid-Depth	1	1	1																
			4_Mid-Surface	1	1	1																
			5_Surface	1	1	1																
			1_Bottom	8.5	2	1	1	1	2	2	2	1	2	1								
			2_Mid-Bottom	2.5	1	1						1		1								
N16	40	A	3_Mid-Depth	10.2	2	2	2	2	2	2	2	2	2	1								
			4_Mid-Surface	2.5	1	1						1		1								
			5_Surface	8.5	2	1	1	1	2	2	2	1	2	1								
			1_Bottom	1	1	1																
			2_Mid-Bottom	1	1	1																

Nearfield Water Column Sampling Plan

StationID	Depth (m)	Station Type	Depths	Total Volume at Depth (L)	Number of 9-L GoFlos	Dissolved Inorganic Nutrients	Dissolved Organic Carbon	Total Dissolved Nitrogen and	Particulate Organic Carbon and	Particulate	Biogenic silica	Chlorophyll a	Total Suspended Solids	Dissolved Oxygen	Rapid Analysis Phytoplankton	Whole Water Phytoplankton	Screened Water Phytoplankton	Zooplankton	Urea	Respiration	Photosynthesis by carbon-14	Dissolved Inorganic Carbon
			Protocol Code			IN	OC	NP	PC	PP	BS	CH	TS	DO	RP	WW	SW	ZO	UR	RE	AP	IC
N17	36	E	3_Mid-Depth	1	1	1																
			4_Mid-Surface	1	1	1																
			5_Surface	1	1	1																
			1_Bottom	15.5	2	1	1	1	2	2	2	1	2							6	1	1
		D+	2_Mid-Bottom	4.5	1	1						1		1							1	1
N18	30	R+	3_Mid-Depth	26.1	3	1	1	1	2	2	2	2	2		1	1	1		1	6	1	2
		P	4_Mid-Surface	4.5	1	1						1		1							1	1
			5_Surface	20.6	2	1	1	1	2	2	2	1	2			1	1		1	6	1	1
			6_Net Tow															1				
N19	24	E	1_Bottom	1	1	1																
			2_Mid-Bottom	1	1	1																
			3_Mid-Depth	1	1	1																
			4_Mid-Surface	1	1	1																
			5_Surface	1	1	1																
N20	32	A	1_Bottom	8.5	2	1	1	1	2	2	2	1	2	1								
			2_Mid-Bottom	2.5	1	1						1		1								
			3_Mid-Depth	10	2	2	1	1	2	2	2	2	2	1								
			4_Mid-Surface	2.5	1	1						1		1								
			5_Surface	8.5	2	1	1	1	2	2	2	1	2	1								
N21	34	E	1_Bottom	1	1	1																
			2_Mid-Bottom	1	1	1																
			3_Mid-Depth	1	1	1																
			4_Mid-Surface	1	1	1																
			5_Surface	1	1	1																
			Totals			111	22	22	42	42	42	42	42	33	1	4	4	2	4	36	10	11
Blanks A									1	1	1	1	1									

Table A-2. Farfield Water Column Sampling Plan (4 Pages). Dissolved Oxygen Quality Assurance Samples will be Collected at the First and Last DO Calibration Station of Each Day.

Farfield Water Column Sampling Plan

StationID	Depth (m)	Station Type	Depths	Total Volume at Depth (L)	Number of 9-L GoFlos	Dissolved Inorganic Nutrients	Dissolved Organic Carbon	Total Dissolved Nitrogen and	Particulate Organic Carbon	Particulate Phosphorous	Biogenic silica	Chlorophyll a	Total Suspended Solids	Dissolved Oxygen	Secchi Disk Reading	Whole Water Phytoplankton	Screened Water Phytoplankton	Zooplankton	Urea	Respiration	Photosynthesis by carbon-14	Dissolved Inorganic Carbon
			Protocol Code			IN	OC	NP	PC	PP	BS	CH	TS	DO	SE	WW	SW	ZO	UR	RE	AP	IC
			Volume (L)			1	0.1	0.1	1	0.3	0.3	0.5	1	1	0	1	4	1	0.1	1	1	1
			1_Bottom	7.9	2	1	1	1	2	2	2	1	2	1								
			2_Mid-Bottom	2.5	1	1						1		1								
F01	27	D	3_Mid-Depth	14	2	1	1	1	2	2	2	2	2	1		1	1		1			
			4_Mid-Surface	2.5	1	1						1		1								
			5_Surface	13	2	1	1	1	2	2	2	1	2	1	1	1	1		1			
			6_Net Tow															1				
			1_Bottom	7.9	2	1	1	1	2	2	2	1	2	1								
			2_Mid-Bottom	2.5	1	1						1		1								
F02	33	D	3_Mid-Depth	15	2	2	1	1	2	2	2	2	2	1		1	1		1			
			4_Mid-Surface	2.5	1	1						1		1								
			5_Surface	13	2	1	1	1	2	2	2	1	2	1	1	1	1		1			
			6_Net Tow															1				
			1_Bottom	1	1	1																
			2_Mid-Bottom	1	1	1																
F03	17	E	3_Mid-Depth	1	1	1																
			4_Mid-Surface	1	1	1																
			5_Surface	1	1	1									1							
			1_Bottom	1	1	1																
			2_Mid-Bottom	1	1	1																
F05	18	E	3_Mid-Depth	1	1	1																
			4_Mid-Surface	1	1	1																
			5_Surface	1	1	1									1							
			1_Bottom	7.9	2	1	1	1	2	2	2	1	2	1								
			2_Mid-Bottom	2.5	1	1						1		1								
F06	35	D	3_Mid-Depth	15	2	2	1	1	2	2	2	2	2	1		1	1		1			
			4_Mid-Surface	2.5	1	1						1		1								
			5_Surface	13	2	1	1	1	2	2	2	1	2	1	1	1	1		1			
			6_Net Tow															1				
			1_Bottom	1	1	1																
			2_Mid-Bottom	1	1	1																
F07	54	E	3_Mid-Depth	1	1	1																
			4_Mid-Surface	1	1	1																
			5_Surface	1	1	1									1							
			1_Bottom	1	1	1																
			2_Mid-Bottom	1	1	1																
F10	30	E	3_Mid-Depth	1	1	1																
			4_Mid-Surface	1	1	1																
			5_Surface	1	1	1									1							
			1_Bottom	4	1	1								3								
			2_Mid-Bottom	2	1	1								1								
F12	90	F	3_Mid-Depth	2	1	1								1								
			4_Mid-Surface	2	1	1								1								

Farfield Water Column Sampling Plan

StationID	Depth (m)	Station Type	Depths	Total Volume at Depth (L)	Number of 9-L GoFlos	Dissolved Inorganic Nutrients	Dissolved Organic Carbon	Total Dissolved Nitrogen and Particulate Organic Carbon	Particulate Phosphorous	Biogenic silica	Chlorophyll a	Total Suspended Solids	Dissolved Oxygen	Secchi Disk Reading	Whole Water Phytoplankton	Screened Water Phytoplankton	Zooplankton	Urea	Respiration	Photosynthesis by carbon-14	Dissolved Inorganic Carbon
			Protocol Code			IN	OC	NP	PC	PP	BS	CH	TS	DO	SE	WW	SW	ZO	UR	RE	AP	IC
			5_Surface	4	1	1								3	1							
			1_Bottom	7.9	2	1	1	1	2	2	2	1	2	1								
			2_Mid-Bottom	2.5	1	1						1		1								
F13	25	D	3_Mid-Depth	15	2	2	1	1	2	2	2	2	2	1		1	1		1			
			4_Mid-Surface	2.5	1	1						1		1								
			5_Surface	13	2	1	1	1	2	2	2	1	2	1	1	1	1		1			
			6_Net Tow															1				
			1_Bottom	1	1	1																
			2_Mid-Bottom	1	1	1																
F14	20	E	3_Mid-Depth	1	1	1																
			4_Mid-Surface	1	1	1																
			5_Surface	1	1	1									1							
			1_Bottom	1	1	1																
			2_Mid-Bottom	1	1	1																
F15	39	E	3_Mid-Depth	1	1	1																
			4_Mid-Surface	1	1	1																
			5_Surface	1	1	1									1							
			1_Bottom	1	1	1																
			2_Mid-Bottom	1	1	1																
F16	60	E	3_Mid-Depth	1	1	1																
			4_Mid-Surface	1	1	1																
			5_Surface	1	1	1									1							
			1_Bottom	1	1	1																
			2_Mid-Bottom	1	1	1																
F17	78	E	3_Mid-Depth	1	1	1																
			4_Mid-Surface	1	1	1																
			5_Surface	1	1	1									1							
			1_Bottom	1	1	1																
			2_Mid-Bottom	1	1	1																
F18	24	E	3_Mid-Depth	1	1	1																
			4_Mid-Surface	1	1	1																
			5_Surface	1	1	1									1							
			1_Bottom	7	2	1														6		
			2_Mid-Bottom	2	1	1								1								
F19	81	F+R	3_Mid-Depth	7	2	1														6		
			4_Mid-Surface	2	1	1								1								
			5_Surface	7	2	1									1					6		
			1_Bottom	1	1	1																
			2_Mid-Bottom	1	1	1																
F22	80	E	3_Mid-Depth	1	1	1																
			4_Mid-Surface	1	1	1																
			5_Surface	1	1	1									1							
			1_Bottom	18	3	1	1	1	2	2	2	1	2							6	1	1
			2_Mid-Bottom	8.5	1	1						1		1							1	2
F23	25	D+R +P	3_Mid-Depth	24	3	1	1	1	2	2	2	2	2			1	1		1	6	1	1
			4_Mid-Surface	7.5	1	1						1		1							1	1
			5_Surface	23	3	1	1	1	2	2	2	1	2		1	1	1		1	6	1	1
			6_Net Tow															1				
			1_Bottom	7.9	2	1	1	1	2	2	2	1	2	1								
			2_Mid-Bottom	2.5	1	1						1		1								

Farfield Water Column Sampling Plan

StationID	Depth (m)	Station Type	Depths	Total Volume at Depth (L)	Number of 9-L GoFlos	Dissolved Inorganic Nutrients	Dissolved Organic Carbon	Total Dissolved Nitrogen and Particulate Organic Carbon	Particulate Phosphorous	Biogenic silica	Chlorophyll a	Total Suspended Solids	Dissolved Oxygen	Secchi Disk Reading	Whole Water Phytoplankton	Screened Water Phytoplankton	Zooplankton	Urea	Respiration	Photosynthesis by carbon-14	Dissolved Inorganic Carbon
			Protocol Code			IN	OC	NP	PC	PP	BS	CH	TS	DO	SE	WW	SW	ZO	UR	RE	AP	IC
F24	20	D	3_Mid-Depth	14	2	1	1	1	2	2	2	2	2	1		1	1		1			
			4_Mid-Surface	2.5	1	1						1		1								
			5_Surface	13	2	1	1	1	2	2	2	1	2	1	1	1	1		1			
			6_Net Tow															1				
			1_Bottom	9.9	2	1	1	1	2	2	2	1	2	3								
			2_Mid-Bottom	2.5	1	1						1		1								
F25	15	D	3_Mid-Depth	15	2	2	1	1	2	2	2	2	2	1		1	1		1			
			4_Mid-Surface	2.5	1	1						1		1								
			5_Surface	15	2	1	1	1	2	2	2	1	2	3	1	1	1		1			
			6_Net Tow															1				
			1_Bottom	1	1	1																
			2_Mid-Bottom	1	1	1																
F26	56	E	3_Mid-Depth	1	1	1																
			4_Mid-Surface	1	1	1																
			5_Surface	1	1	1									1							
			1_Bottom	7.9	2	1	1	1	2	2	2	1	2	1								
			2_Mid-Bottom	2.5	1	1						1		1								
F27	108	D	3_Mid-Depth	15	2	2	1	1	2	2	2	2	2	1		1	1		1			
			4_Mid-Surface	2.5	1	1						1		1								
			5_Surface	13	2	1	1	1	2	2	2	1	2	1	1	1	1		1			
			6_Net Tow															1				
			1_Bottom	1	1	1																
			2_Mid-Bottom	1	1	1																
F28	33	E	3_Mid-Depth	1	1	1																
			4_Mid-Surface	1	1	1																
			5_Surface	1	1	1									1							
			1_Bottom	2	1	1								1								
			2_Mid-Bottom	2	1	1								1								
F29	66	F	3_Mid-Depth	2	1	1								1								
			4_Mid-Surface	2	1	1								1								
			5_Surface	2	1	1								1	1							
			1_Bottom	9.9	2	1	1	1	2	2	2	1	2	3								
			3_Mid-Depth	14	2	1	1	1	2	2	2	2	2	1		1	1		1			
F30	15	G	5_Surface	15	2	1	1	1	2	2	2	1	2	3	1	1	1		1			
			6_Net Tow															1				
			1_Bottom	9.9	2	1	1	1	2	2	2	1	2	3								
			3_Mid-Depth	14	2	1	1	1	2	2	2	2	2	1		1	1		1			
F31	15	G	5_Surface	15	2	1	1	1	2	2	2	1	2	3	1	1	1		1			
			6_Net Tow															1				
F32	30	Z	5_Surface												1							
			6_Net Tow															1				
F33	30	Z	5_Surface												1							
			6_Net Tow															1				
			1_Bottom	8.1	2	1	2	2	2	2	2	1	2	1								
			2_Mid-Bottom	2.5	1	1						1		1								
N16	40	D	3_Mid-Depth	15	2	2	2	2	2	2	2	2	2	1		1	1		1			
			4_Mid-Surface	2.5	1	1						1		1								
			5_Surface	13	2	1	1	1	2	2	2	1	2	1	1	1	1		1			
			6_Net Tow															1				
			Totals			132	35	35	66	66	66	62	66	76	28	22	22	13	22	36	5	6

Farfield Water Column Sampling Plan

StationID	Depth (m)	Station Type	Depths	Total Volume at Depth (L)	Number of 9-L GoFlos	Dissolved Inorganic Nutrients	Dissolved Organic Carbon	Total Dissolved Nitrogen and	Particulate Organic Carbon	Particulate Phosphorous	Biogenic silica	Chlorophyll a	Total Suspended Solids	Dissolved Oxygen	Secchi Disk Reading	Whole Water Phytoplankton	Screened Water Phytoplankton	Zooplankton	Urea	Respiration	Photosynthesis by carbon-14	Dissolved Inorganic Carbon
			Protocol Code			IN	OC	NP	PC	PP	BS	CH	TS	DO	SE	WW	SW	ZO	UR	RE	AP	IC
Blanks B									1	1	1	1	1									
Blanks C									1	1	1	1	1									
Blanks D									1	1	1	1	1									

Appendix B

Comparison of Three Secchi Disks

Memorandum

To: Mike Mickelson, Harbor Studies, ENQUAD

From: Dave Taylor, Central Lab, ENQUAD

Subject: Comparison of Secchi Disk measurements

Date: April 30, 1998

cc: S. Cibik (ENSR), C. Hunt (Battelle), Kelly Coughlin (MWRA)

Attached is data from Don Boye' (ENSR) comparing readings from 3 types of secchi disks at 25 stations in Boston Harbor and Mass. and Cape Cod bays. This 3 types of disks encompass the range of types of disks employed by recent monitoring programs in Boston Harbor, and Mass. And Cape Cod bays.

To recap, the HOM and BHWQM programs have in the past employed a variety of secchi disks. The HOM program has employed 12 " all white oceanographic disks and 8" black and white quadrant disks. BHWQM has employed 8" all white and 8" quadrant disks.

For the 25 stations on these dates, the differences between the 3 types of disks was small relative to the precision of each of the methods. The 12" all-white disk yielded an average ($\pm$ S.D.) depth only 1.09 $\pm$ 0.07 m greater than the 8" all-white disk. The 8" all white quadrant disk yielded an average depth only 1.05 $\pm$ 0.08 m greater than the 8" quadrant disk.

These differences are smaller than the difference Kelly and I observed when we compared 8" all-white and 8" quadrant disks in the Inner Harbor. We got a difference of 1.2 $\pm$ 0.08 m difference, perhaps as a result of the humic stained waters in the Inner Harbor. Perhaps in stained fresh or brackish water such as these, the effects of different types of disks may be larger.

For unstained waters, such as those of the Outer Harbor and Mass and Cape Cod bays, the changes in types of disk employed across programs is likely to have affected the secchi disk measurements by 10% or less. I am struck by how small this effect is, considering the relatively large differences in white area among disks. The 8" all-white disk has 2x more white than the 8" quadrant disk. A 12" all-white disk has 2.25 x more white than the 8" all white disk, and 4.48 x more white than the 8" quadrant disk.

Table B-1. Comparing Results Obtained from an All White Oceanographic Secchi Disk and the Standard Black and White Quadrant Secchi Disk

Secchi Depth Recorded (meters)			
Station ID	**12" Oceanographic (All White)**	**8" Oceanographic (All White)**	**8" Standard (Black/White)**
F01	8.50	8.00	N/A
F02	9.00	7.50	N/A
F12	14.50	13.50	N/A
F27	12.00	12.00	N/A
F26	8.00	8.50	N/A
N07	11.00	10.50	10.50
N04	5.50	5.50	5.50
F18	4.50	4.00	4.00
N01	6.50	6.00	5.00
N10	4.50	4.00	4.00
F25	4.50	4.00	4.00
F31	4.00	3.50	3.50
F30	2.75	2.25	2.25
F23	3.50	3.00	3.00
F24	4.00	3.75	3.75
N16	4.50	4.00	4.00
F17	11.00	11.00	10.50
F16	11.00	11.00	11.00
F15	7.00	6.50	6.50
F13	7.00	6.50	6.50
F14	4.00	3.50	3.00
F07	7.00	6.50	5.50
F06	6.50	6.00	5.00
F05	7.00	6.00	5.50
F03	6.50	6.00	5.50

Appendix C

A Brief Comparison of Digestion Vessels for Total Dissolved Nitrogen and Phosphorus Determinations

A Brief Comparison of Digestion Vessels for Total Dissolved Nitrogen and Phosphorus Determinations.

By Carolyn Keefe
Chesapeake Biological Laboratory
University of Maryland Center for Environmental Science
Ref. No. [UMCES]CBL 98-062
March 3, 1998

Introduction

Since 1978 the Nutrient Analytical Services Laboratory at University of Maryland's Chesapeake Biological Laboratory has been simultaneously determining total dissolved nitrogen and phosphorus concentrations by alkaline persulfate digestion, then measuring colorimetrically the resultant nitrate and phosphate concentrations by continuous flow analysis. Until summer 1990 we employed the method of D'Elia *et al*. (1977), performing numerous comparisons between sample concentrations of total dissolved nitrogen determined by alkaline persulfate oxidation and total Kjeldahl acid digestion; and between total dissolved phosphorus by alkaline persulfate digestion and acid persulfate digestion (Menzel and Corwin, 1965). The data were always comparable, although persulfate digestion for nitrogen yielded more precise replication than Kjeldahl determination (*e.g.*, D'Elia *et al*., 1987). The convenience of analysis of one digestate for simultaneous determination of total dissolved nitrogen and phosphorus led to the employment of the alkaline persulfate method for both determinations. The method, in summary, depends on alkaline conditions at the start of the digestion phase which are suitable for the nitrogen compounds' quantitative oxidation to nitrate. As the decomposition of the persulfate continues, the solution becomes acidic, and, thus suitable for the phosphorus compounds' oxidation to orthophosphate.

In the summer of 1990, we exhausted a case of sodium hydroxide (NaOH) that we had used for over a decade to make up the alkaline potassium persulfate reagent for each digestion. At that point, we were surprised to find a persistently high total dissolved phosphorus blank, no matter which supplier of NaOH was employed. We compared our method with others and noticed some subtle differences in NaOH concentration, so we eventually settled on reducing the concentration of NaOH to one third of that of D'Elia *et al*. (1977) to reduce the blank level. On the basis of grams of NaOH in the persulfate reagent per volume of sample, we add approximately the same amount of NaOH as Valderrama (1981) CBL .0015g NaOH/ml sample; Valderrama .0019g NaOH/ml sample. It may be important to the question of silicate release from glass that the CBL persulfate reagent is made up as a more dilute solution and then a larger quantity is added to the sample CBL 10 ml sample + 5 ml persulfate reagent; Valderrama 30ml sample + 4 ml persulfate reagent.

In brief, we now add 5 ml of freshly made persulfate reagent (20.1 g $K_2S_2O_8$ and 3.0 g NaOH in 1 L deionized water) to 10 ml of sample in a 30 ml screw cap culture tube with a polypropylene linerless cap, then digest in a pressure cooker at 100-110 C and 3-4 psi for 60 minutes. After cooling to atmospheric temperature and pressure, 1 ml borate buffer (61.8 g boric acid and 8 g sodium hydroxide in 1 L deionized water) is added. The samples are then analyzed for nitrate and orthophosphate.

After the necessary change in methodology, there was no step in the data trends of nitrogen or phosphorus for any long term data set that we analyzed. We still found low concentrations (by difference) of dissolved organic phosphorus in most oxic and anoxic areas of the Chesapeake and its tributaries in any season. Dissolved organic phosphorus concentrations ranged generally from 0 to 0.5 umoles/L.

Dissolved organic phosphorus concentrations remained higher in the vicinity of sewage treatment plants and some blackwater rivers on Maryland's Eastern Shore.

It is possible that the glass digestion tubes employed at CBL could be contributing an interference due to the measurement of silica released from the alkaline breakdown of the borosilicate glass during the digestion. If this were to be occurring, it might be more significant in the most saline samples since those are among the most alkaline samples that we encounter. The resultant high silica concentrations could be erroneously measured as orthophosphate by the phosphomolybdate blue technique employed. To investigate this potential problem, we prepared samples and standards made up in two different salinity waters and deionized water. These were then digested simultaneously in borosilicate culture tubes and Teflon bottles. They were analyzed colorimetrically for the resultant nitrate and orthophosphate digestion products.

Materials and Methods

Thirty ml screw cap culture tubes were cleaned by digesting them with persulfate digestion reagent, then rinsing with deionized water. Thirty ml screw cap Teflon bottles were cleaned by soaking in 1 N HCl, then rinsing with deionized water.

Standards were made up in deionized water and 10 ml added to 30 ml screw cap borosilicate glass culture tubes and 30 ml screw cap Teflon bottles. Samples of filtered Sargasso Sea water (36 ppt salinity, 0.03 umoles orthophosphate-P/L, <0.02 μmoles nitrate+nitrite-N/L) and filtered Chesapeake Bay water (9.7 ppt salinity, 0.19 μmoles orthophosphate-P/L, 21.7 μmoles nitrate +nitrite-N/L) were added to culture tubes and Teflon bottles. Sargasso Sea water and Chesapeake Bay water were spiked with primary standard grade KNO_3 and KH_2PO_4, then apportioned and added to culture tubes and Teflon bottles. Deionized water, Sargasso Sea water and Chesapeake Bay water were spiked with reagent grade glutamic acid and glycerophosphate, then apportioned and added to culture tubes and Teflon bottles. Ten ml of deionized water were added to culture tubes and Teflon bottles as deioinized water blanks. Empty (so-called reagent blank) tubes and bottles were also prepared. All samples, standards and reagent blanks in the tubes and bottles were prepared in triplicate. Five ml persulfate oxidation reagent (20.1 g $K_2S_2O_8$ and 3 g NaOH/L) were added to each tube or bottle, then shaken and all were placed in the pressure cooker together. After digestion for 1 hr at 100-110 C, the tubes and bottles were brought back to room temperature over the period of 1 hr. One ml borate buffer was added to each with shaking. The standards, reagent blanks and samples were analyzed for nitrate and orthophosphate colorimetrically. Ten ml of deionized water (containing no detectable nitrate+nitrite or orthophosphate) were added to the reagent blanks with shaking just before analysis.

The output from the colorimeter was plotted as a function of the concentration of the standards made up in distilled water, including the tubes or bottles containing 10 ml deionized water that had been digested. The resultant standard curve for those in Teflon bottles was used to calculate concentrations of saline samples or saline samples spiked with nitrate, orthophosphate, glycerophosphate or glutamic acid, or deionized water spiked with glycerophosphate or glutamic acid that had been digested in Teflon. The resultant standard curve for those in borosilicate glass tubes was used to calculate concentrations of saline samples or saline samples spiked with nitrate, orthophosphate, glycerophosphate or glutamic acid, or deionized water spiked with glycerophosphate or glutamic acid that had been digested in glass.

The reagent blanks and y intercepts for dissolved phosphorus were virtually identical for digestions carried out in the same vessels, *e.g.*, the glass reagent blank was virtually identical to the glass y intercept, etc.. Thus, there was no significant phosphorus present in the deionized water used to make up the standards. The reagent blanks for dissolved nitrogen were slightly lower than the corresponding y intercepts. This revealed that there was probably dissolved nitrogen in the deionized water used to make

up the standards. Therefore, to obtain the most accurate measurement of nitrogen concentration in the saline samples and saline spiked samples, the value of the corresponding (glass or Teflon) reagent blank was subtracted from the raw reading. The resultant corrected value was then divided by the appropriate (glass or Teflon) slope to obtain the dissolved nitrogen concentration.

The mean and standard deviation of each triplicate sample subset were calculated.

Results

The concentrations of all subsets comparing Teflon to glass digestion vessels were very similar. The reagent blanks and y intercepts of those digested in glass were slightly higher than those digested in Teflon. The total dissolved phosphorus concentrations of saline samples (36 and 9.7 ppt) digested in Teflon and determined from the standard curve generated by Teflon-digested standards were identical to those digested in glass and determined from the standard curve generated by glass-digested standards. The total dissolved nitrogen concentrations of saline samples digested in Teflon and determined from the standard curve generated by Teflon-digested standards were within the Method Detection Limit (MDL) of those digested in glass and determined from the standard curve generated by glass-digested standards. Results are summarized in Tables C-1 and C-2.

"Parallel" concentrations of saline samples and spiked saline samples digested in Teflon compared to those digested in glass were always within the MDL of the determinations. In some corresponding pairs, the concentrations were within the standard deviations of the triplicate measurements. Measured concentrations of spiked Sargasso Sea water and Chesapeake Bay water samples were all within expectations of the sums of the inorganic spikes added and the respective component water's original concentration. Measured concentrations of glycerophosphate and glutamic acid spiked in deionized water, Sargasso Sea water and Chesapeake Bay water were all within expectations of the sums of their components, considering that the compounds added were reagent grade rather than the more accurate primary standard grade available for the inorganic spikes.

It is important to note that the reagent blank and y intercept of dissolved phosphorus digested in glass was only the equivalent of 0.06 μmoles of phosphorus greater than those digested in Teflon. Obviously, the impact of this slightly elevated glass reagent blank and *y* intercept was accounted for in the calculation of concentrations with the appropriate standard curves. The potential impact of increased release of interfering silicate on the concentrations of the most saline samples determined from glass digestion vessels was not found.

Table C-1. Total Dissolved Phosphorus Results		
	Digested in Glass Tubes	Digested in Teflon Bottles
Reagent Blank	2.3±0.40 SD chart units	1.2±0.10 SD chart units
KH_2PO_4 Standards in deionized Slope	19.400	19.022
Intercept	2.20 chart units	1.11 chart units
R	.9994	.9999
Sargasso Sea water	0.28 ±0.026 μmoles P/L	0.28±0.009 μmoles P/L
Chesapeake Bay water	0.40±0.024 μmoles P/L	0.40±0.000 μmoles P/L
3 μmoles KH_2PO_4/L in Sargasso Sea water	3.17±0.004 μmoles P/L*	3.19±0.017 μmoles P/L*
3 μmoles KH_2PO_4/L in Chesapeake Bay water	3.24±0.036 μmoles P/L*	3.33±0.016 μmoles P/L*
6 μmoles KH_2PO_4/L in Sargasso Sea water	6.09±0.078 μmoles P/L*	6.10±0.024 μmoles P/L*
6 μmoles KH_2PO_4/L in Chesapeake Bay water	6.16±0.066 μmoles P/L*	6.24±0.018 μmoles P/L*
3.95 μmoles P/L glycerophosphate in deionized water	4.05±0.044 μmoles P/L	4.06±0.027 μmoles P/L
3.95 μmoles P/L glycerophosphate in Sargasso Sea water	4.19±0.003 μmoles P/L*	4.30±0.025 μmoles P/L*
3.95 μmoles P/L glycerophosphate in Chesapeake Bay water	4.30±0.044 μmoles P/L*	4.38±0.014 μmoles P/L*
Method Detection Limit (previously determined)	0.041 μmoles P/L	
*The concentration listed includes the background dissolved phosphorus present in the filtered saline water.		

Table C-2. Total Dissolved Nitrogen Results

	Digested in Glass Tubes	Digested in Teflon Bottles
Reagent Blank	4.1±0.57 chart units	3.0±0.12 chart units
KNO_3 Standards in deionized Slope	1.4451	1.4013
Intercept	5.13 chart units	4.38 chart units
R	.9991	.9998
Sargasso Sea water	7.9±1.25 μmoles N/L	6.5±0.80 μmoles N/L
Chesapeake Bay water	43.7±0.57 μmoles N/L	44.2±0.50 μmoles N/L
25 μmoles KNO_3/L in Sargasso Sea water	34.2±1.07 μmoles N/L*	31.6±0.19 μmoles N/L*
25 μmoles KNO_3/L in Chesapeake Bay water	64.7±1.12 μmoles N/L*	67.0±0.45 μmoles N/L*
50 μmoles KNO_3/L in Sargasso Sea water	58.3±0.93 μmoles N/L*	56.6±0.50 μmoles N/L*
50 μmoles KNO_3/L in Chesapeake Bay water	87.3±3.27 μmoles N/L*	88.3±0.04 μmoles N/L*
46 μmoles N/L glutamic acid in deionized water	47.1±0.74 μmoles N/L	46.3±0.36 μmoles N/L
46 μmoles N/L glutamic acid in Sargasso Sea water	56.3±0.86 μmoles N/L*	55.1±1.39 μmoles N/L*
46 μmoles N/L glutamic acid in Chesapeake Bay water	83.3±0.74 μmoles N/l*	84.9±0.36 μmoles N/L*
Method Detection Limit (previously determined)	1.75 μmoles N/L	

*The concentration listed includes the background dissolved nitrogen present in the filtered saline water.

Conclusion

There is no significant source of contamination to total dissolved nitrogen or total dissolved phosphorus samples digested in borosilicate glass digestion tubes when compared to concentrations determined in Teflon bottles. When determining concentrations of samples, it is better to calculate concentrations from standards digested in vessels of the same material.

Literature Cited and Other Related References

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Appendix D

Comparative Evaluation of Total Suspended Solids in Sea Water Measured Using Glass Fiber, Suprapore Polyethersulfone, and Nuclepore Polycarbonate Filters

BACKGROUND

Various types of filters (*e.g.*, glass fiber, Nuclepore polycarbonate membranes, Suprapore polyethersulfone membranes) have been used to measure total suspended solids in effluent, aquatic systems (lakes, stream, rivers, and seawater). Each type of filter has characteristics that make them more or less desirable depending on the media and goals of the measurement program. For example, GF/F have generally been used in standard methods for waste and wastewater due to the high flow rates and ability to pass large volumes before clogging. On the other hand, GF/F have the disadvantage of having relatively high tare mass (thus requiring high TSS loads to overcome measurement variability) and variable pore size (GF/F filters generally used for environmental measurements have nominally 0.7 μm openings). In contrast, Nuclepore membranes have much lower mass (allowing loading of relatively small mass particles) and extremely consistent pore size (0.4 or 0.45 μm pore openings). According to generally accepted convention and EPA regulatory definitions that this pore size defines the difference between dissolved and particulate matter. However, Nuclepore membranes have low tortuosity, thus plug quickly when small, uniform particles are present in the sample (often occurs when samples are dominated by small, nondiatomaceous phytoplankton). This limits the sample volume that can be filtered. Nuclepore filters are also prone to developing static charges. Suprapore filters are intermediate in mass and thicker, providing greater filtration volumes than Nuclepore filters and are not as prone to static buildup.

The water types listed above are characterized by distinctly different salt content at the macroscale and high variability on short term temporal or spatial scales within water type, especially in the coastal zone. This fact is key to proper selection of membrane type for TSS measurements. In the mid 1970's, marine scientists working in coastal systems recognized that GF/F tended to give high TSS levels relative to TSS measured using membranes. Unpublished evaluations suggested that GF/F gave unacceptable TSS measurements in sea water which were generally ascribed to high blank values. The cause of the high relative TSS results and blanks were generally unknown but were suspected to be caused by salt retention, water retention, or other factors. These factors were not generally pursued nor published. Over time, scientists conducting dissolved metals measurements in coastal waters discontinued the use of glass fibers to remove particles from the sample and migrated to membrane filters as the filter of choice. This development was not so much due to inconsistencies in TSS measurements but to the need and ability to control unwanted introduction of metal contamination during processing.

TSS in receiving waters monitored under the Harbor and Outfall Monitoring program have been measured using a variety of filter types. From 1992 through 1994 Nuclepore filters (0.4 μm pore size) were used to determine TSS, in 1995 GF/F were used. From 1996 through 1997, TSS measurements were made using Nuclepore (0.45 μm). During the mid-1990's, Battelle began to use 0.4 μm Suprapore filters to determine TSS in coastal waters, where TSS is relatively high. The Suprapore filters were used due to ease of use and ability to pass large volumes of water thereby providing higher loads on the filters which lead to better precision and lower detection limits. The ability of these several filter types to provide comparable TSS results has been questioned in the past but never fully tested. In particular, GF/F have been questioned regarding their ability to provide accurate TSS results in waters with high salt content.

Battelle proposed to use Suprapore filters in the HOM3 monitoring program. However, the need to have highly comparable data in the HOM program and varied history of TSS methods under the monitoring program demanded that an intercomparison study of various filter types be conducted. This appendix reports the result of such an intercomparision, completed by Battelle under rigorous method protocols and conducted by experienced analysts. The results are presented in three sections 1) study design, 2) results and discussion, 3) and conclusions and recommendations.

STUDY DESIGN

Two experiments were conducted with the express goal of demonstrating that GF/F gave erroneously high results relative to membrane filters when used to measure TSS in sea water and to document the comparability of the Suprapore and Nuclepore filters for performing TSS measurements in fresh and salt waters. Battelle SOP 5-053 *(Suspended Particulate Matter Measurements (Total Suspended Solids [TSS]*) was followed for these comparisons. The first experiment was designed to directly compare TSS levels in a coastal sea water sample using three filter types. For this test, TSS values were determined from a single primary sample of Duxbury Harbor sea water sampled on January 26, 1998. Three precleaned and tared filter types (47-mm diameter GF/F glass fiber, 47-mm diameter 0.4 micron Nuclepore, and 47-mm diameter 0.4 micron Suprapore) were used. Seven replicates of the Duxbury Bay sea water were filtered for each filter type. The filtered volume of each replicate was 250 mL. Samples were not randomly aliquoted among the 21 filters. Rather, all Nuclepore replicates were taken before pouring the seven Suprapore replicates followed by the seven glass fiber replicates. The primary sample was gently swirled before pouring of each aliquot. While it is possible that the procedures could have allowed a gradient in particle concentrations to develop in the primary sample bottle due to settling, it is unlikely. In addition, a set of deionized water blanks were passed through two additional Nuclepore membranes. Blanks were not developed for the other two filter types. After the sample was passed through the filter, each filter was rinsed three times with deionized water adjusted to pH 8 to remove sea salt and allowed to air dry for at least 24 hours before weighing to determine the mass of particles on the filter. Internal QC included weighing at least one filter of each type in triplicate for both the tare and loaded (different filter than tared triplicate) filters. The precision of these replicated individual filter tare weighing was 0.015493 $\pm$ 0.000049 (CV = 0.32%), 0.071093 $\pm$ 0.000032 (CV = 0.05%), and 0.127077 $\pm$ 0.000023 (CV = 0.018%) for the Nuclepore, Suprapore, and GF/F, respectively (see attached data tables for individual weighting results). Similar levels of individual filter weighting precision were found for loaded filters (see attached data tables).

The second experiment was developed from the results of the first experiment. This experiment was specifically designed to evaluate whether differences in filtration blanks for "particle-free" (defined below) sea water and deionized water filtered through each of the three types of filters could be detected. For this experiment, 2 liters of sea water from Duxbury Harbor was sequentially filtered trough Nuclepore, Suprapore and GF/F to generate "particle-free" water. Particle levels and size distributions were not checked with independent instrumentation, *i.e.*, particle counters. To conduct the experiment, an equal volume of deionized water (Millipore 18 ΩM) from the Battelle trace metals laboratory was also sequentially filtered as above. The "particle-free" sea and deionized waters were then filtered in triplicate using cleaned and tared filters of each filter type. One hundred milliliters of filtered water was used for each sample. Filtrations were done randomly among the three filter types for each water type. Each filter was rinsed three times with pH 8 deionized water, air dried, weighed, and the TSS blank of each replicate calculated in mg/L.

RESULTS AND DISCUSSION

Experiment 1

The mean tare weight (in grams) of the three filter types increased from 0.01504 $\pm$ 0.00006; CV = 0.39% (Nuclepore) to 0.071354 $\pm$ 0.00197; CV =2.8% (Suprapore) to 0.126336 $\pm$ 0.00169; CV = 1.34% (glass fiber) (See attached experiment 1 data table). The tare weight of the GF/F is 8.2 times more than the Nuclepore filters and 1.77 times more than Suprapore filters. Suprapore filters are 4.7 times heavier than the Nuclepore filters. Precision among seven replicates of each filter type decreased from Suprapore to glass fiber to Nuclepore filters. Evaluation of the change in mass that could be detected on each filter type using the precision of triplicate tare weight of the filters indicates that changes in mass in the fifth

decimal place (0.00005 g) can be observed for each filter type. This evaluation using the mean mass and standard deviation of seven individual filters suggest changes in mass on the filters must be in the fourth decimal place for the Nuclepore filters (0.00009 g) and third decimal place for the Suprapore and GF/F (0.002 g) to detect incremental increases due to loading on the filters. Thus, to ensure accurate measurement of TSS the filters must be loaded with large amounts of particles. For example, if a sample has 1 mg TSS/L, a value typical of Massachusetts Bay, at least one liter and more likely two or more liters would have to be filtered to measure TSS levels in the sample using Suprapore and GF/F. In contrast, the Nuclepore filters will provide detectable levels of TSS if as little as 250 mL is filtered.

The method detection limit for TSS can be calculated based on the weighing error of filters and volume of sample to be filtered. For example, for triplicate weighing of a single Nuclepore filter, the precision estimate as the CV was found to be 0.32 and 0.24 percent before and after loading, respectively (Table D-1). Assuming the higher CV represents the weighing error, the minimum change in filter weight that can be detected can be determined by multiplying the tared filter mass by the CV. Using the mean tare weight of seven individually tared filters (Table D-1) to obtain the most representative mass of clean filters, this value can be estimated as

$$0.01531 \text{ g} \times 0.0032 = 0.00005 \text{ g } (0.05 \text{ mg}).$$

If this value is divided by the volume filtered (*e.g.*, 0.5 L) the detectable TSS concentration can be estimated, for example, as

$$0.05 \text{ mg}/0.5 \text{ L} = 0.1 \text{ mg/L}$$

If volumes other than 500 mL are filtered, the detectable TSS concentration would vary as follows:

0.05 mg/L @ 1,000 mL filtered
0.2 mg/L @ 250 mL filtered
0.5 mg/L @ 100 mL filtered.

If a more conservative approach is taken by multiplying the minimum detectable change of an individual filter by 3, the estimated method detection limit becomes

0.15 mg/L @ 1000 mL filtered
0.3 mg/L @ 500 mL filtered
0.6 mg/L @ 250 mL filtered
1.5 mg/L @ 100 mL filtered

If the same process is followed for the glass fiber and Suprapore filters, the MDLs for filtering 500 mL of water through these filter types become 0.1 and 0.2 mg/L @500 mL filtered, respectively.

This assessment demonstrates the importance of understanding the required sample volume to filter relative to each filter type. Regardless it is always desirable to filter the maximum amount of water possible to increase the accuracy and precision of the TSS measurements, especially when other factors such as blank control and natural variability are considered.

Examination of the mean TSS concentrations measured by the seven replicates of each of the three filter types clearly shows levels above the MDL. Also evident in the data are higher values for the glass fiber treatment relative to both Nuclepore and Suprapore. TSS determined using Suprapore filters gave results that were about 50% higher than measured by Nuclepore (ratio 1.4) while use of GF/F resulted in approximately 60% higher TSS relative to the Nuclepore filters (ratio 1.6). Further, the precision of the

TSS measurement reported as the percent CV increased between the Nuclepore (CV = 7%), Suprapore (15%), and glass fiber (18%) filters (Table D-1).

Experiment 2

As found in Experiment 1, the determination of the tare weight of six individual filters of each filter type was very precise at 1.1, 2.5, and 0.65% as the coefficient of variation for Nuclepore, Suprapore, and GF/F, respectively. The tare weights of the six filters of each type were very close to those measured in the first experiment (compare mean tare weights in Experiment 1 [Table D-1] and Experiment 2, [Table D-2]).

The passage of 100 mL of the filtered deionized water through the filters provided surprising results. Triplicate deionized water blanks filtered through Nuclepore filters showed a small loss of mass (-0.6 ± 0.44 mg/L). The Suprapore and GF/F demonstrated an even larger loss of mass in this experiment -1.5 ± 0.29 and -2.1 ± 0.55 mg/L, respectively.

In contrast, passage of 100 mL of filtered sea water through individual filters showed the opposite response. The Nuclepore filters lost slight lower amounts of mass (-0.23 ± 0.76 mg/L) than with deionized water, while the Suprapore and GF/F showed TSS concentrations of 3.7 ± 0.16 and 8 ± 1.9 mg/L, respectively. These values are very high compared to the expected result of 0.0 mg/L and that determined by using the Nuclepore filters. For each filter type, the coefficient of variation for the triplicate sets were high-ranging from 20 to 43 percent for glass fiber and Suprapore filters and even higher values for the Nuclepore. The Nuclepore CV may be expected to be higher as it is derived from data very close to the measurement limits for filters.

The potential for water retention was checked by drying selected filters for one hour at 70 °C and reweighing. Weight changes were not observed. Thus, the increase in filter mass noted after the filtration of particle-free seawater was likely due to factors other than water retention, most likely salt retained within the membranes or glass fibers.

CONCLUSIONS AND RECOMMENDATIONS

TSS measured in sea water was lowest and most precise when Nuclepore filters were used. An evaluation of the causes of the low relative values in the Nuclepore filters focused on whether the other filters had high blanks. Evaluation of whether or not retention of particles was less effective for Nuclepore was not conducted due to the results of the blank studies.

The study showed that

> Passage of filtered deionized water through glass fiber and Suprapore filters gave negative filter blanks,
>
> Passage the filtered deionized water through Nuclepore filters gave blanks values that were close but slightly less than zero.
>
> Filter sea water passed through glass fiber and Suprapore filters resulted in high TSS blank values with GF/F having the highest relative blank value.
>
> Nuclepore filters had a low but variable sea water TSS blank values.

The study demonstrated that GF/F can not be used to accurately determine natural levels of TSS in coastal waters. Similarly, even though Suprapore filters give lower blanks relative to glass fiber, the blank is

unacceptably high for coastal water TSS determination. The measured blank using Suprapore filters equals many reported TSS values from coastal and estuarine systems. Only Nuclepore filters gave seawater blanks that were near zero.

The data also suggests that GF/F may loose weight when deionized water is passed through the filter. This raises concerns regarding the accuracy of low level TSS measurements using GF/F in fresh water systems. Further examination of this phenomena relative to treatment plant effluent measurements and in aquatic systems should be conducted. Such studies should focus on the cause of the blank variability and appropriate volumes of sample required to achieve measurement accuracy.

These data indicate that among the three filter types evaluated only Nuclepore filters can provide consistently low TSS blanks. The use of this filter as the filter membrane of choice is recommended for the MWRA HOM program. Data generated by other filter types must be considered suspect. Similar comparative studies should be conducted if other filter types have been used to measure TSS in the Harbor and in Massachusetts Bay to these filter types to provide accurate measures of TSS.

Lastly, estimates of the MDL for TSS measurements show that practical detection limits vary as a function of volume of water filtered. For the HOM3 program, which will filter a nominal value of 500 mLs of seawater, a TSS MDL of 0.1 mg/L is estimated. Due to the positive and negative deviations in the variability of the measurements used to derive this value slight negative values may be occasionally found in the samples and in method blanks. These should be evaluated carefully to determine if they are consistent with the information developed in this study.

Table D-1. Experiment 1

Battelle Duxbury Operations
Project Name: Comparison Study
Project Number: C1013181-22
Batch Number: 98-09
Entered By: JIL 01/26/98 JIL 01/29/98 RH 1/29/98
Filename: Filter Test TSS

Filter ID	Weighed By: Initials/Date	Tare Weight (g)	QC check 1	QC check 2	Mean Tare Weight (g)	Battelle Sample ID	Sample Filtered	Weighed By: Initials/Date	Gross Weight (g)	QC check 1	QC check 2	Mean Gross Weight (g)	TSS (g)	TSS (mg/L)	Mean TSS (mg/L)
TESTN001	JIL 01/26/98	0.01534	Nuclepore		0.01534	LF07-R#1	250	JIL 01/29/98	0.01682			0.01682	0.00148	5.92	
TESTN002		0.01540	Nuclepore		0.01540	LF07-R#2	250		0.01719			0.01719	0.00179	7.16	
TESTN003		0.01521	Nuclepore		0.01521	LF07-R#3	250		0.01704			0.01704	0.00183	7.32	
TESTN004		0.01531	Nuclepore		0.01531	LF07-R#4	250		0.01717	0.01719	0.01711	0.01716	0.001846667	7.386666667	
TESTN005		0.01532	Nuclepore		0.01532	LF07-R#5	250		0.01704			0.01704	0.00172	6.88	
TESTN006		0.01529	Nuclepore		0.01529	LF07-R#6	250		0.01705			0.01705	0.00176	7.04	
TESTN007		0.01527	Nuclepore		0.01527	LF07-R#7	250		0.01702			0.01702	0.00175	7	6.958095238
TESTN008		0.01538	Nuclepore		0.01538	LF08-R#1	250		0.01534			0.01534	-4E-05	-0.16	
TESTN009		0.01555	0.01546	0.01547	0.01549	LF08-R#2	250		0.01540			0.01540	-9.33333E-05	-0.373333333	-0.266666667
TESTS001		0.06953	Suprapore		0.06953	LF07-R#8	250		0.07226			0.07226	0.00273	10.92	
TESTS002		0.07240	Supropore		0.07240	LF07-R#9	250		0.07459			0.07459	0.00219	8.76	
TESTS003		0.07156	Supropore		0.07156	LF07-R#10	250		0.07379			0.07379	0.00223	8.92	
TESTS004		0.07475	Supropore		0.07475	LF07-R#11	250		0.07670			0.07670	0.00195	7.8	
TESTS005		0.07150	Supropore		0.07150	LF07-R#12	250		0.07411			0.07411	0.00261	10.44	
TESTS006		0.06867	Supropore		0.06867	LF07-R#13	250		0.07165			0.07165	0.00298	11.92	
TESTS007		0.07107	0.07113	0.07108	0.07109	LF07-R#14	250		0.07333	0.07341	0.07338	0.07337	0.00228	9.12	9.697142857
TESTG001		0.12305	Glass Fiber		0.12305	LF07-R#15	250		0.12593			0.12593	0.00288	11.52	
TESTG002		0.12622	Glass Fiber		0.12622	LF07-R#16	250		0.12856			0.12856	0.00234	9.36	
TESTG003		0.12547	Glass Fiber		0.12547	LF07-R#17	250		0.12788			0.12788	0.00241	9.64	
TESTG004		0.12674	Glass Fiber		0.12674	LF07-R#18	250		0.12933			0.12933	0.00259	10.36	
TESTG005		0.12785	Glass Fiber		0.12785	LF07-R#19	250		0.13052			0.13052	0.00267	10.68	
TESTG006		0.12793	Glass Fiber		0.12793	LF07-R#20	250		0.13102			0.13102	0.00309	12.36	
TESTG007		0.12709	0.12705	0.12709	0.12708	LF07-R#21	250		0.13087	0.13083	0.13087	0.13086	0.00378	15.12	11.29142857

Decimal place for measurement change

Tare	Gross
0.00005	0.00004
0.00003	0.00004
0.00002	0.00002

Precision of triplicate weighting of tare and loaded filters

	Tare				Loaded		
Filter Type	Mean (g)	STD (g)	CV %		Mean (g)	STD (g)	CV %
Nuclepore	0.01549	0.00005	0.318		0.01716	0.00004	0.243
Supropore	0.07109	0.00003	0.045		0.07337	0.00004	0.055
Glass Fiber	0.12708	0.00002	0.018		0.13086	0.00002	0.018

7 replicated filters	TSS g/L	Ratio TSS/Tare
5.91E-05	4.91E-04	8.31E+00
1.97E-03	1.44E-03	7.30E-01
1.69E-03	1.98E-03	1.17E+00

Mean Tare Weight of Seven Filters

Filter Type	Mean (g)	STD (g)	CV %
Nuclepore	0.01531	0.00006	0.39
Supropore	0.07135	0.00197	2.76
Glass Fiber	0.12634	0.00169	1.34

Ratio	
S/N	4.66
G/N	8.25
G/S	1.77

Measured TSS by three filters, n = 7

Mean (mg/L)	STD (mg/L)	CV %	
6.96	0.49	7.1	S/N
9.70	1.44	14.8	G/N
11.29	1.98	17.5	G/S

Table D-2. Experiment 2

Battelle Duxbury Operations

Project Name: Comparison Study

Project Number: C1013181-22

Batch Number: 98-11

Entered By: JIL 02/02/98 /26/98

Filename: Filter Test TSS ;./dsa

Filter ID	Weighed By: Initials/Date	Tare Weight (g)	QC check 1	QC check 2	Mean Tare Weight (g)	Battelle Sample ID	Sample Filtered	Weighed By: Initials/Date	Gross Weight (g)	QC check 1	QC check 2	Mean Gross Weight (g)	TSS (g)	TSS (mg/L)	Mean TSS (mg/L)
TEST2N001	RB 2/02/98	0.01512	Nuclepore		0.01512	LF16-FBR#1	100	RB 2/13/98	0.01503	Deionized		0.01503	-0.00009	-0.90	
TEST2N002		0.01550	Nuclepore		0.01550	LF16-FBR#2	100		0.01549	Deionized		0.01549	-0.00001	-0.10	
TEST2N003		0.01526	Nuclepore		0.01526	LF16-FBR#3	100		0.01518	Deionized		0.01518	-0.00008	-0.80	-0.60
TEST2N004		0.01503	Nuclepore		0.01503	LF17-SWR#1	100		0.01509	Salt H2O		0.01509	0.00006	0.60	
TEST2N005		0.01517	Nuclepore		0.01517	LF17-SWR#2	100		0.01513	Salt H2O		0.01513	-0.00004	-0.40	
TEST2N006		0.01515	Nuclepore		0.01515	LF17-SWR#3	100		0.01506	Salt H2O		0.01506	-0.00009	-0.90	-0.23
TEST2S001		0.07074	Suprapore		0.07074	LF16-FBR#4	100		0.07061	Deionized		0.07061	-0.00013	-1.30	
TEST2S002		0.06801	Suprapore		0.06801	LF16-FBR#5	100		0.06783	Deionized		0.06783	-0.00018	-1.80	
TEST2S003		0.07318	Suprapore		0.07318	LF16-FBR#6	100		0.07305	Deionized		0.07305	-0.00013	-1.30	-1.47
TEST2S004		0.07129	0.07131	0.07129	0.07130	LF17-SWR#4	100		0.07154	0.07152	0.07151	0.07152	0.00022	2.18	
TEST2S005		0.07234	Suprapore		0.07234	LF17-SWR#5	100		0.07269	Salt H2O		0.07269	0.00035	3.53	
TEST2S006		0.07166	Suprapore		0.07166	LF17-SWR#6	100		0.07219	Salt H2O		0.07219	0.00053	5.30	3.66
TEST2G001		0.12830	Glass Fiber		0.12830	LF16-FBR#7	100		0.12806	Deionized		0.12806	-0.00024	-2.40	
TEST2G002		0.12656	Glass Fiber		0.12656	LF16-FBR#8	100		0.12631	Deionized		0.12631	-0.00025	-2.50	
TEST2G003		0.12768	Glass Fiber		0.12768	LF16-FBR#9	100		0.12753	Deionized		0.12753	-0.00015	-1.50	-2.13
TEST2G004		0.12773	Glass Fiber		0.12773	LF17-SWR#7	100		0.12861	Salt H2O		0.12861	0.00088	8.80	
TEST2G005		0.12670	Glass Fiber		0.12670	LF17-SWR#8	100		0.12763	Salt H2O		0.12763	0.00093	9.30	
TEST2G006		0.12616	Glass Fiber		0.12616	LF17-SWR#9	100		0.12674	Salt H2O		0.12674	0.00058	5.80	7.97

Mean Tare Weight of Six Filters

Filter Type	Mean (g)	STD (g)	CV %
Nuclepore	0.015205	0.000163	1.07
Suprapore	0.071263	0.001779	2.50
Glass Fiber	0.127188	0.000832	0.65

	Ratio
S/N	4.68
G/N	8.36
G/S	1.79

	Sea water				Deionized Water	
Filter Type	Mean (mg/L)	STD (mg/L)	CV %		Mean (mg/L)	STD (mg/L)
Nuclepore	-0.23	0.76	-327.3		-0.60	0.44
Suprapore	3.66	1.56	42.7		-1.47	0.29
Glass Fiber	7.97	1.89	23.8		-2.13	0.55

Decimal place for measurement change

Tare		TSS g/L	Ratio (TSS/Tare)
0.00016	Nuclepore	7.64E-04	4.76E+00
0.00178	Suprapore	1.56E-03	8.79E-01
0.00083	Glass fiber	1.89E-03	2.28E+00

Appendix E
Primary Production by ^{14}C

In order to justify changing the incubation for ^{14}C primary production from 75 ml polycarbonate incubation containers to 20 ml borosilicate scintillation vials, a series of comparisons will be needed. The advantage of the 20 ml containers is that direct counts for C-14 can be made on the vial after acid addition and after the scintillation cocktail has been added, eliminating the error-prone filtration step of larger volume incubations.

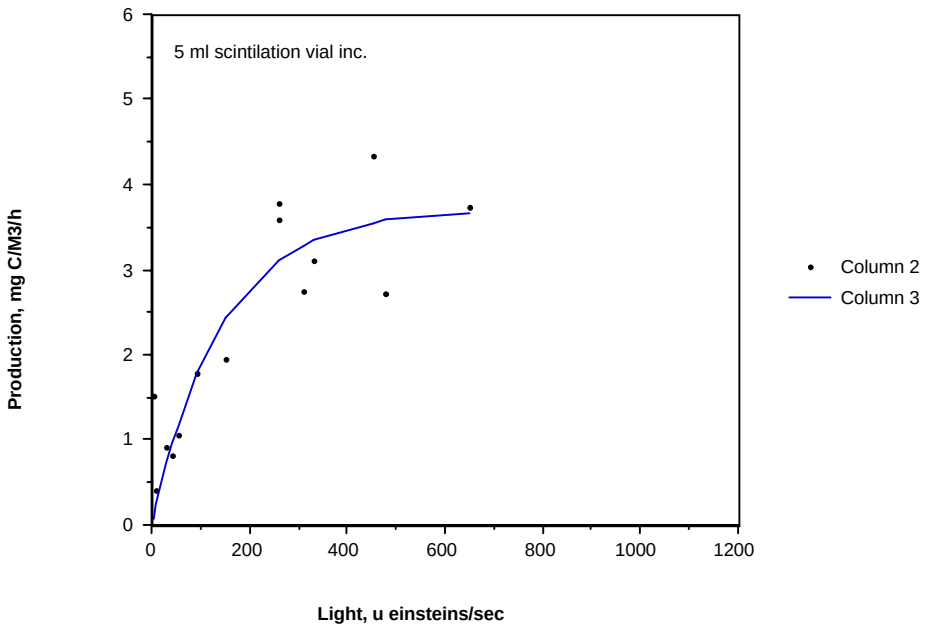
To date, two series of comparisons have been made to reveal similarities and differences between the two methods. In January 1998 the 5 ml in the 20 ml vial incubation was compared to the 300 ml BOD incubation (Fig. 1). For both P_{max} and *alpha*, there were no significant differences between the two methods at the 0.05 level (Table E-1). In December 1997 a 300 ml glass bottle, a 60 ml glass bottle, and a 75 ml polycarbonate bottle were compared in a natural light gradient over depth in a Marine Ecosystem Research Laboratory (MERL) enclosure (Fig. 2). For both P_{max} and *alpha* there were no significant differences at the 0.05 level (Table E-1). Both of these comparisons thus support the use of the small volume (5 ml) in the 20 ml vial.

Table E-1. Comparisons of 5 mL versus 300 mL container type, and container type and volume for C-14.

Volume	Incubation		X	95%
Comparison of 5 ml and 300 ml Incubations, January 1998:				
5 ml	Scintillation vial in light box	P_{max}	3.70	3.00-4.40
		$_{x}$	0.03	0.01-0.04
300 ml	BOD bottle in enclosure	P_{max}	3.86	3.43-4.30
		$_{x}$	0.05	0.03-0.60
Comparison of 300 ml, 60 ml, and 75 ml and Incubations, December 1997				
300 ml	BOD bottle in Enclosure	P_{max}	1.66	1.55-1.77
		$_{alpha}$	0.03	0.03-0.04
60 ml	BOD bottle in Enclosure	P_{max}	1.5	1.31-1.68
		$_{alpha}$	0.04	0.03-0.06
75 ml	Polycarbonate bottle in Enclosure	P_{max}	1.66	1.61-1.71
		$_{alpha}$	0.04	0.03-0.05

Figure E-1. A comparison of 5 ml scintillation vial measures of *alpha* and P_{max} and 300 ml BOD bottle measures for C-14 primary production incubations. A) 5 ml in 20 ml vial in light incubation box. B) 300 ml BOD bottle in a natural light gradient over depth in a MERL enclosure.

1A



1B

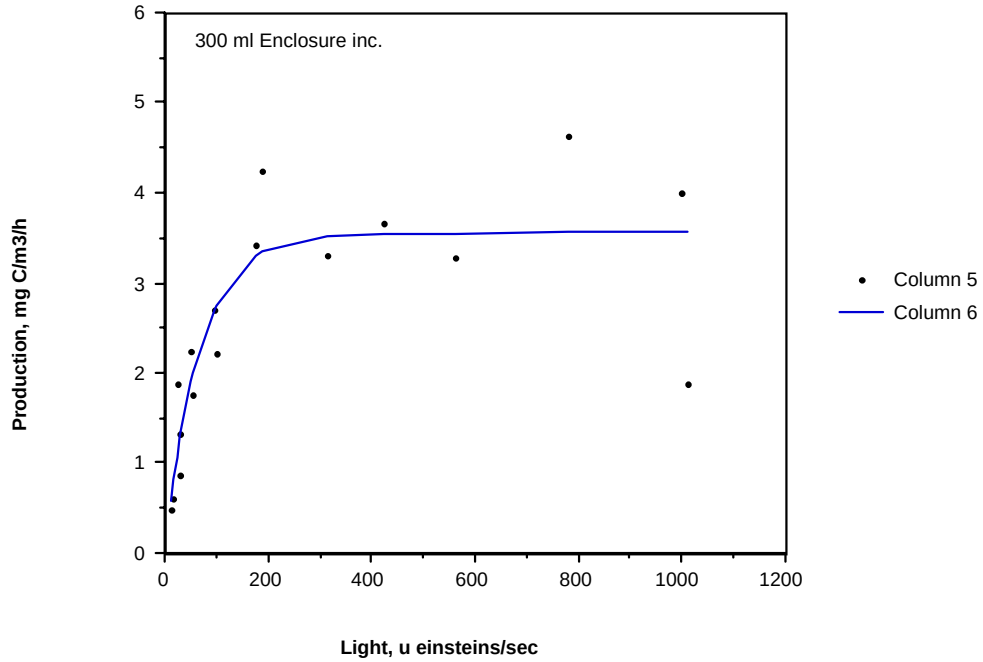
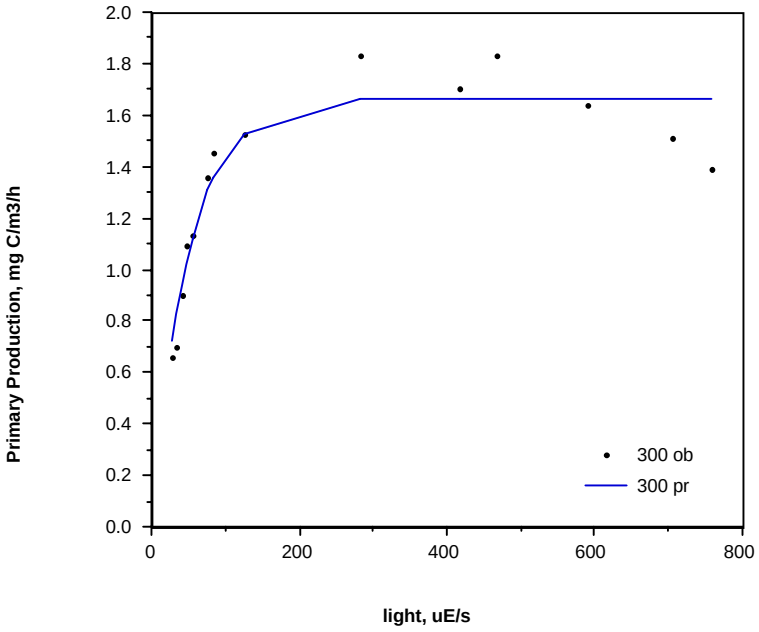
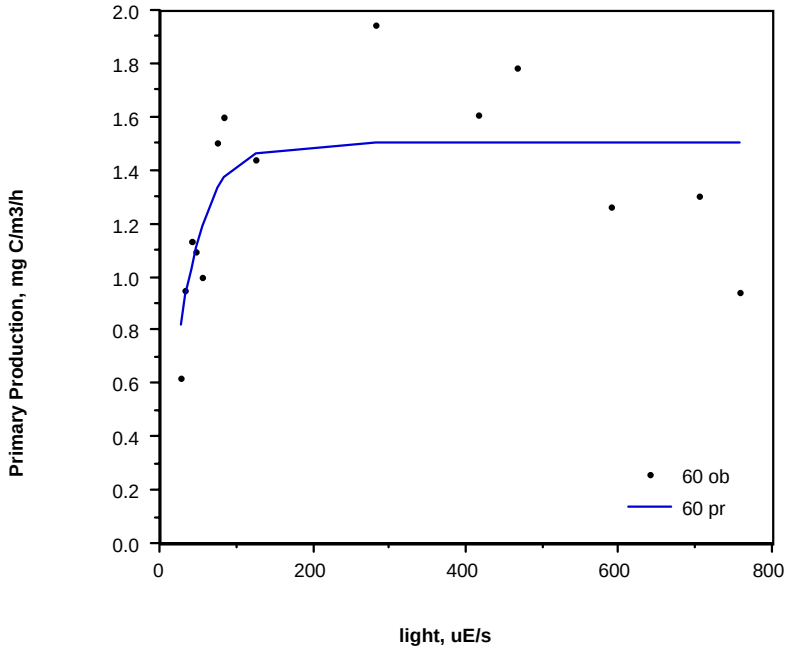


Figure E-2. Comparisons of container size and type on C-14 primary production incubations. A) 300 ml BOD bottle; B) 60 ml BOD bottle; and C. 75 ml polycarbonate bottle in natural light gradients over depth in a MERL enclosure.

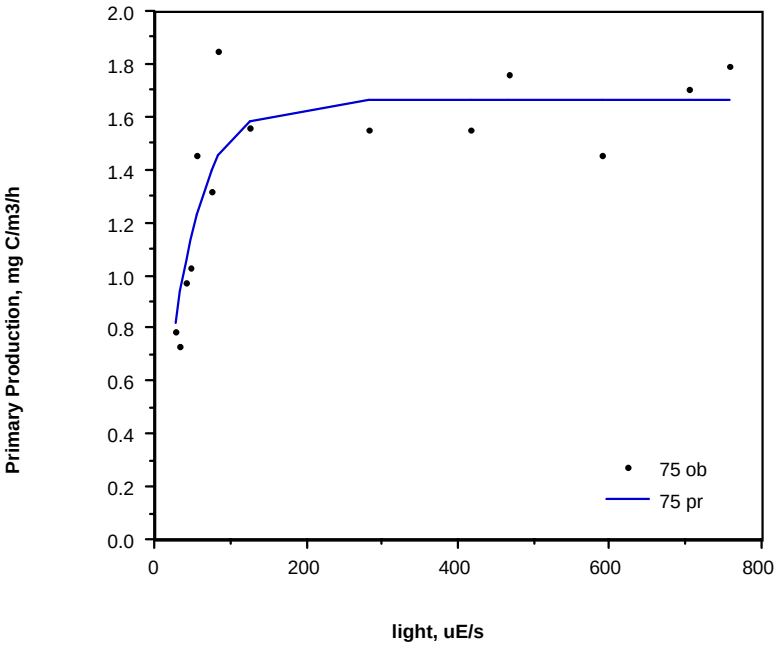
2A



2B



2C


Primary Production, mg C/m3/h
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
200
400
600
800
light, uE/s
75 ob
75 pr

Appendix F

Whole-Water Phytoplankton Analysis Comparison Study

MEMORANDUM

TO: Carlton Hunt

FROM: Don Anderson

SUBJECT: Phytoplankton QA comparisons

DATE: **A**pril 13, 1998

Introduction

The HOM3 project team includes several contractors which are different from the ones involved in HOM2. With regard to phytoplankton community composition analysis, HOM2 counts were performed at the Philadelphia Academy of Sciences (ANS)by Richard Lacouture, whereas those for HOM3 were conducted at the University of Massachusetts, Dartmouth UMD) by David Borkman. (Throughout this text, UMD means D. Borkman, and ANS means R. Lacouture). In addition to the change in personnel, the procedures used to settle and concentrate the samples differed between the two laboratories, as did the type of counting chamber and microscope. Given legitimate concerns about the comparability of methods and identification procedures, a series of comparison counts of natural and simulated field samples was set up as a result of discussions between C. Hunt, M. Mickelson, D. Anderson and S. Cibik. Separate tests were designed to address settling efficiencies, microscope optics, and species identification. This memo summarizes these comparison efforts.

Experimental Design

Table F-1 lists the three main types of comparisons that were conducted. Details of the procedures used to create these samples are given in the Methods section, but the distinction between procedures can be summarized as follows. Samples with the prefix SET were intended to assess settling efficiencies using mixed laboratory cultures of picoplankton. The SETUMD series involved settling 1 liter samples in 1-liter graduated cylinders, and then gently aspirating away the supernatant to give 25mL of concentrate. These were counted at UMD by D. Borkman. SETWH samples were those for which 50 mL were settled in an inverted microscope chamber, leaving a concentrate of 2.4 mL to be counted at the Woods Hole Oceanographic Institution on an inverted microscope, again by D. Borkman. SETUMD-1W, 2W, and 3W were 2.4 mL samples of the original 25-ml concentrate from SETUMD, placed in an inverted microscope chamber and counted at WHOI on the inverted microscope by Borkman. SETANS were 50 mL samples settled in an inverted microscope chamber, leaving a concentrate of 2.4 mL to be counted at the ANS by R. Lacouture.

Samples with the prefix ID were intended to compare the taxonomic identifications of the two contractors. The material examined was collected in Eel Pond, Woods Hole. ID-UMD samples were settled in an inverted microscope chamber from 50 mL and counted at WHOI on the inverted microscope by Borkman, whereas the ID-ANS series were concentrated in a similar fashion and counted at the ANS by Lacouture.

The MWRA -A,B,C,D, E series were samples collected during the Harbor Outfall Monitoring Program, settled from 50 mL to 2.4 by the ANS, and from 100 mL to 10 mL at UMD. The former were counted by Borkman using an inverted microscope at WHOI, and the latter using a compound microscope with long working distance objectives at UMD. As a further check of the settling process, samples with the prefix EQ were counted, representing 2.4 mL from the 25 mL UMD concentrate of MWRA-A,B,C,D.

Methods

Sample preparation. Two samples were prepared on December 15, 1997. The first sample was made by combining 4 laboratory cultures, one diatom and three prymnesiophytes, such that the cell concentrations would approach natural densities of nano/pico-plankton known to occur in Massachusetts Bay; approximately 100,000-500,000 cells per liter. The four phytoplankton cultures chosen were: Clone 3HTP or CCMP1335, *Thalassiosira pseudonana* a 4-6 µm diatom; clone EH, *Emiliania huxelyi*, a 6 µm (prymnesiophyte; clone CCMP 1325, *Pavlova lutheri*, a 3-6 µm prymnesiophyte; and clone T. ISO, *Isochrysis* sp. a 3-6 µm prymnesiophyte.

1.5 mL aliquots of each culture were preserved with Utermöhls fixative and then counted with a Fuchs Rosenthal counting chamber at a total magnification of 400X to determine cell density. Two of the cultures, 3HTP and EH, required a 1:11 dilution in filtered seawater prior to counting due to their high density. The entire 256 gridded area of the chamber was counted in duplicate for each culture (approximately 200-600 cells/count). Initial concentrations of each clone were as follows: 3HTP; 1.77×10^6 mL^{-1}, EH; 2.06×10^6 mL^{-1}, CCMP1325; 1.35×10^5 mL^{-1}, T. ISO; 5.86×10^5 mL^{-1}. Based on these counts, each culture originally preserved for the Fuchs Rosenthal counts, was added to 3.5 L of 0.2 µm (filtered Vineyard Sound seawater containing 100 g Utermöhls solution, such that the following densities were achieved: 3HTP; 2.00×10^5 L^{-1}, EH; 5.00×10^5 L^{-1}, CCMP1325; 4.00×10^4 L^{-1}, T. ISO; 1.75×10^4 L^{-1}. The volumes of each culture added were: 3HTP; 395 (L, EH; 850 (L, CCMP1325; 1040 (L and T. ISO; 1050 (L. From this 3.5 liter sample 3-1 liter aliquots were dispensed into 1 L polyethylene bottles and delivered to J. Turner, U-Mass Dartmouth (samples SETUMD-1,2,3). These samples were to be settled and counted at U-Mass Dartmouth following their standard protocols. When the counts of these samples were completed, the remaining material was transported to Woods Hole and 2.4 mL was added to Utermöhls counting chambers and settled for a minimum of 2 hours and counted again by D. Borkman (samples SETUMD-1W,2W,3W). Also, 3-125 mL samples were added to 125 mL polyethylene bottles and retained at WHOI for settling in 50 mL Utermöhls chambers for at least 48 hours after which they were counted by D. Borkman (samples SETWH-1,2,3). An additional set of 3-125 mL samples was mailed to ANS for enumeration (samples SETANS-1,2,3). The original 3.5 L sample was gently but thoroughly mixed prior to dispensing each subsample.

To address differences in species identification between labs, a second sample was made by collecting 4 liters of water with a bucket from the inlet to Eel Pond, Woods Hole which was preserved with 100 mL. Utermöhls solution. The sample was well mixed and dispensed into six 125 mL plastic bottles. Three of these bottles were mailed to ANS (samples ID-ANS-1,2,3) and the remaining 3 samples were kept at WHOI to be settled in 50 mL Utermohls chambers for at least 48 hours and counted by D. Borkman (samples ID-UMD-1,2,3).

Samples EQ-1,2,3,4 were originally samples MWRA-A,B,C,E, from the HOM2 outfall monitoring program. These were received from the ANS and sent to D. Borkman at UMD (100 mL each). After concentration and counting on 1 mL at UMD, the left-over sample was delivered by J. Turner to WHOI and 2.4 mL were added to Utermohls counting chambers and settled for 24 hours prior to being counted on the inverted microscope by D. Borkman.

All counts performed by D. Borkman at WHOI were on a Zeiss IM35 inverted microscope equipped with 12.5X oculars and a 40X plan objective (total magnification = 500X).

ANS Procedures. Lacouture used Leitz Diavert inverted scopes for the ANS phytoplankton counts. The oculars used were either 10X or 12.5X depending on the particular microscope. The objectives used were 25/0.50 and 40/0.70 (magnification/numerical aperture), and the numerical aperture of the condensers was 0.70. Total magnification ranged from 250 - 500X.

The counting technique begins by settling an appropriate subsample of the whole water sample. The volume of this subsample for the MWRA samples is either 10, 25 or 50 mL. The gravitational settling takes place in a 2-piece, inverted microscope (Utermohls) settling chamber. Settling times vary according to the height of the column - the 10-mL column settles for at least 12h, the 25-mL column for at least 24h and the 50-mL column for at least 48h. Upon settling the column is removed from the bottom plate which holds ~2.4 mL (the concentrated subsample).

The cell enumeration procedure consists of a dual magnification examination of the samples, whereby cells generally < 5 µm are identified and counted at the high magnification (500X). The dominant at this magnification was inevitably “unidentified microfglagellates”. Individual cells of this dominants are counted until a raw count of 75 of the top dominant is attained and a minimum of 20 random fields for the other small forms. This is followed by a low magnification count at 312.5X, in which 75 individuals of the top 3 dominants were tabulated. The counting program will not allow counts of a particular taxon at more than one magnification, so there is no repetition within counts.

UMD Procedures. Counts are made at UMD on an Olympus BH-1 conventional light microscope equipped with 10X eyepieces, 10, 20 and 40X long working distance objective lenses and a 1.25X magnification dichroic mirror/fluorescence illuminator, resulting in total magnifications of 125, 250 and 500X. (Only 500 and 250X are used for counts). Phase contrast illumination is used.

For concentration, small samples (volume ca. 100 mL) were placed in a 100 mL graduated cylinder (27 mm ID X 188 mm height) and allowed to settle for 5 days. Larger samples (up to 1000 mL) are placed in a 1-l graduated cylinder and settled for approximately one week, after which all but 25 mL are siphoned off. After the settling period, samples are decanted by

siphoning off the upper 90 mL of fluid (from the 100-mL samples) or 975 mL (from the 1000 mL samples). Siphoning was done using a length of ca. 5 mm ID rubber tubing into which a standard borosilicate-glass Pasteur pipette was inserted. This pipette was taped to the end of a plastic rod (ca. 75 mm long) which served as a handle for lowering the pipette, with siphon hose attached, into the settled sample. The tip of the pipette was placed below the surface of the settling sample and a siphon was started by drawing on the opposite end of the tube with a pipette bulb. Once the sample fluid entered the siphon tube, and a siphon was started, the far end of the tube was placed in a sink that was about 0.5 m lower than the counter top that the settling sample was on. The supernatant sample fluid was then allowed to flow, via gravity siphon, out of the settling tube and into the sink. As the fluid drained, the pipette tip was kept just below the meniscus of the settling fluid by gradually lowering the pipette (taped to the plastic handle) synchronous with the rate of fluid level drop. The material remaining after siphoning was swirled (by hand) for about one minute and transferred to a 50 mL plastic sample tube. Sample concentration was nominally 10:1 and sometimes as high as 40:1.

Counting is done in a 1 mL Sedgwick-Rafter cell at 500X magnification. The chamber is divided into 48 equal paths, and random paths are scanned at 500X until 400 cells of the dominant taxa are seen. Greater than 400 dominant-taxa cells were often counted as the 400-cell criterion was usually reached mid-path and counting had to be continued until the end of that path. After the 500X count, a scan of the entire chamber is made at 250X to look for larger phytoplankton cells not encountered in the 500X count.

Results

Settling tests (the SET series). The first test was to designed to determine if the contractors could generate comparable results when counting a mixture of laboratory cultures preserved in Lugols iodine, and if a single contractor could generate comparable counts using two different settling procedures and microscopes (Table F-2). This experiment revealed several problems inherent in preserving and counting picoplankton, especially those which are small and fragile - identification to the species level is difficult, and cells are easily lost, perhaps through cell lysis. The original intention was to have samples with 200,000, 500,000, 40,000, and 17,500 cells L^{-1} of *Thalassiosira pseudonana, Emiliania huxleyi, Pavlova lutheri,* and *Isochrysis* sp., but the results suggest that neither contractor was able distinguish *E. huxleyi* from *P. lutheri*. Therefore, those counts are combined for this analysis.

Both laboratories agreed reasonably well with each other on the species counts, although both consistently undercounted compared to the computed cell densities. Counts done on the four species contained in the SET samples by both D. Borkman and R. Lacouture were substantially lower than expected, especially for *E. huxelyi*. For this species only 35 percent of the cells, at best, were counted by either individual regardless of the settling method or microscope used. To verify Borkman's counts, sample SETWH-1 was quickly re-counted by D. Kulis (50 fields at 500X) to be certain that the relatively small *E. huxelyi* were present and could be accurately enumerated. D. Kulis found the sample to be somewhat difficult to count in that there was a significant amount of debris in the sample even though safeguards were taken to minimize this during sample preparation (see Methods). Regardless, his counts were similar to those of D. Borkman for this sample and he was convinced that *E. huxelyi*, for some unknown reason, was

present in much lower numbers than calculated. Several possibilities for causing the disappearance of this species were investigated. These included:

- *The salinity of the seawater used for the dilution of the 3.5 L sample was significantly different than that of the culture medium these isolates were originally cultured in*. However, measurements showed that the salinity of the diluent was about 32 ppt., the same as the seawater used for the cultures. There thus was no osmotic shock.

- *E. huxelyi cells were not being efficiently settled*. To test this possibility, the supernatant for the SETWH-1 sample (approximately 47 mL after settling and removal of the bottom chamber) was concentrated, via filtration, onto a 0.8-µm-pore-size Nuclepore filter. A drop of propidium iodide was placed on top of the filter to stain the nuclei of any cells which may have been caught by the filter. No cells were observed on the filter upon examination by epifluorescence microscopy, so there were none in the supernatant. A control sample of *E. huxelyi* was processed and stained in a similar fashion and the nuclei of those cells were easily observed on the filter.

- *Losses occurred in the settling chimneys.* To verify that losses don't occur when a sample is settled in a Utermohls counting chimney and slide (*e.g.*, cell adhesion to the settling chimney), 2.4 mL of the original SETWH-1 sample was added to an inverted counting chamber and allowed to settle over 72 hours before enumerating. These results were similar to D. Kulis' SETWH-1 counts.

- *Errors were made in the original cell counts.* To verify that an error was not made on the original cell count used to determine the cell density of the *E. huxelyi* culture (a 1:11 dilution of preserved EH culture) the same sample was recounted on January 28th as previously described. This count gave a density of only 3.68×10^5 mL^{-1}, almost an order of magnitude less than the December 15th count of 2.06×10^6 mL^{-1}. This discrepancy suggests that either the first count was in error, or cells had lysed in the 1:11 diluted sample. The undiluted EH culture was again diluted 1:11 in filtered seawater and recounted. Here cell densities of 2.38×10^6 mL^{-1} were tallied which is very similar to the count performed on this sample on December 15. The dramatic decrease in cell values in the original 1:11 dilution indicates that cells had burst or lysed during the interval after they were diluted, but that the undiluted sample was unaffected during that same time.

In summary, the disappearance of *E. huxelyi* cells in this repeat of the dilution process suggests that the cells which were spiked into the 3.5 L SET sample also suffered a similar fate. Why these cells disappeared remains a mystery, but at least some insight has been gleaned as to why both D. Borkman and R. Lacouture counted far fewer microflagellates in these samples than anticipated.

Looking to other issues in the SET series, the precision within each series of counts was generally good by both contractors, with the UMD coefficient of variation ranging from 2 to 27% for abundant organisms, and from 27-49% for ANS. This difference between ANS and UMD reflects the larger number of cells counted by UMD. Although both labs were given the same criteria for counting, UMD often counted 3-4X more cells for each.

UMD settled these samples two different ways, and used two different microscopes for counting, but there was no systematic difference between the counts (Table F-2). This argues that the 1000-mL settling procedure did not lose cells due to insufficient settling time, convection currents, or wall effects. Likewise, the resolution obtained with long-working distance objectives and a Sedgewick Rafter slide was comparable to that obtained with standard objectives looking from below through a thin cover glass into an inverted microscope (Utermohls) settling chamber.

Species Identification (the ID series). In Table F-3, the counts of a freshly-collected and preserved sample from Eel Pond, Woods Hole are compared, again with two different settling and counting options for UMD. Dominant counts are presented in Table F-3. First, comparing the UMD counts to each other, there is a hint that the counts using the inverted microscope are higher than those with the 1 liter settling column and the compound scope for the smaller forms (microflagellates, *Cryptomonas, Guinardia)* but the differences are not large, and except for the microflagellates, not significantly different). Microflagellate counts by ANS were approximately 1/2 to 1/3 of the UMD counts, but other counts were generally comparable. It is possible that the lower microflagellate counts reflect loss of these small cells during shipment or extended storage.

Species Identification (the MWRA series). Table F-4 lists the dominant species in 5 Massachusetts Bay samples collected during the MWRA Harbor Outfall Monitoring Program, again with two different settling and counting procedures for UMD. Comparing the two UMD counts to each other first, no obvious or systematic differences are apparent. Differences are apparent between UMD and ANS, however. With the exception of Sample A, UMD counts of microflagellates are consistently lower than the ANS counts, typically by a factor of 2-3. This difference with smaller forms is also seen in the counts of *Cryptomonas* species, which again are either extremely low in the UMD counts, or not detected at all. Clearly, if the *Cryptomonas* counts are added to the microflagellate totals, the discrepancy between UMD and ANS is even larger. The UMD counts using the inverted microscope at WHOI were on material identical to that counted by UMD on the compound microscope at UMD. Since the microflagellate counts on samples A-E using the inverted were actually lower than the compound microscope counts of the exact same, settled material, the difference is not one of optics, but rather must be due to either loss of cells during the settling process or during transportation. The original samples had all been sent to ANS as part of the HOM2 program. Subsamples were sent back to WHOI and then to UMD where they were settled. In addition to these discrepancies between the counts for the small species, there were also differences in the counts of a number of the larger species, such as *Skeletonema costatum* or *Leptocylindrus danicus*.

Conclusions

The original objective of this study was to determine if there was any problem with either methods or taxonomic identifications associated with a change in phytoplankton contractors between HOM2 and HOM3. A series of comparative counts were organized, some with natural samples, and some with mixed laboratory cultures. The results show considerable variability between laboratories, but the differences are not always systematic or easily attributed to a single factor. A few issues are of note.

1) MWRA HOM3 samples counted by D. Borkman with two different microscopes were consistently lower in microflagellates than the ANS counts. This is not an optical problem, but instead relates to either settling methods or to losses of small cells during prolonged storage. Similarly, samples of the ID mailed to ANS resulted in counts that were 1/3 to 1/2 as high as UMD counts, again suggesting a loss of cells with time and distance traveled.

Recommendation: It would be worthwhile to have UMD count a sample repeatedly through time over a month or more to ascertain the extent to which storage losses occur. This could be accomplished using one of the HOM3 samples, with WHOI archiving the sample and giving it to D. Borkman on a recurrent basis, without him knowing that it is the same sample If differences are observed through time, very strict counting and storage guidelines should be instituted by the MWRA.

2. It is not possible to conclude from these data that the UMD compound microscope with long working distance objectives and the Sedgewick Rafter slide give results consistently higher or lower than an inverted microscope using Utermohls chambers. Nevertheless, the scrutiny that will be given to MWRA procedures when it is time to determine if "significant change" in the phytoplankton community has occurred once the outfall begins operation suggests that advocating both a change in personnel and a change in settling and counting methods will be criticized.

Recommendation: Additional counts should be performed in parallel by D. Borkman during the routine HOM3 surveys. Selected samples should be counted first using the standard UMD methods for settling and counting, and then again (blind) using an inverted microscope with Utermohls chambers. If sufficient samples are counted in this manner by the same individual, it will either provide the data needed to argue that the change in methods is not a significant problem or will demonstrate that all subsequent HOM3 cell counts should be performed using inverted microscopes and Utermohls chambers.

3. Some significant differences were observed in the identification of key species or groups of phytoplankton (*e.g.* cryptomonads and even some large diatoms).

Recommendation: An effort should be made for D. Borkman and R. Lacouture to spend time together so that reference keys and other literature can be standardized, and so that the categories used in HOM2 can be carried though into HOM3. For example, the ANS has typically broken several groups of organisms into size fractions (*i.e.* unidentified centric diatoms <10 µm, and unidentified centric diatoms 10-30 µm), but UMD does not. UMD should make

every effort to recreate the ANS categories. Other groups that should be standardized include the cryptomonads, *Pseudo-nitzschia, Phaeocystis,* and microflagellates in general.

4. There are conflicting reports in the literature about the size of settling columns, but several publications are adamant that the smallest phytoplankton species cannot be settled in columns in which the ratio of height to diameter exceeds 5. (Some argue that it is not possible to settle any sample larger than 50 mL effectively).

Recommendation: All HOM3 samples should be settled in columns in which the ratio of height to diameter does not exceed 5. If large (i.e., 800-1,000 mL) containers are used, at least a week of settling time must be allowed and draft-free conditions should be provided during settling.

Table F-1. Details of comparison count samples

Sample ID	Sample Origin	Laboratory	Settling Method	Counting Method
SETUMD-1,2,3	Mixed culture	UMD	1 liter to 25 mL in triplicate	1 mL Sedgwick Rafter, cmpd. scope 500X
SETWH-1,2,3	Mixed culture	WHOI	50 mL to 2.4 mL in triplicate	Inverted scope, 500X
SETUMD-1W,2W,3W	Mixed culture	WHOI	2.4 mL from remaining SETUMD-1,2,3	Inverted scope, 500X
SETANS-1,2,3	Mixed culture	ANS	50 mL to 2.4 mL in triplicate	Inverted scope, 500X
ID-URI-1,2,3	Eel Pond, Woods Hole	WHOI	50 mL to 2.4 mL in triplicate	Inverted scope, 500X
ID-ANS-1,2,3	Eel Pond, Woods Hole	ANS	50 mL to 2.4 mL in triplicate	Inverted scope, 500X
MWRA-A,B,C,D	MWRA	ANS	unknown	Inverted scope, 500X
MWRA-A,B,C,D	MWRA	UMD	~120 mL to 10 mL in triplicate	1 mL Sedgwick Rafter, cmpd. scope 500X
EQ-1,2,3,4	MWRA samples A,B,C,E	WHOI	2.4 mL from remaining MWRA-A,B,C,E	Inverted scope, 500X

Table F-2. Mixed Picoplankton Cultures – average of Triplicate counts (cells/L ±SD)

Species		Concentration (cells/L ±SD and CV)			
		UMD			ANS
Volume settled (mL)		1000 mL	1000 mL	50 mL	50 mL
Analysis method		Sedgwick-Rafter	Inverted	Inverted	Inverted
Analysis location		UMD	WHOI	WHOI	ANS
Thalassiosira pseudonana (200,000 cells/L)	Mean	62,918	101,992	119,818	122,260
	SD	6,547	3,389	2,190	32,789
	CV	10.4%	3.3%	1.8%	26.8%
Emiliania huxleyi and Pavlova lutheri* (540,000 cells/L)	Mean	133,033	95,100	146,998	7,858
	SD	30,084	1,371	18,080	3,881
	CV	27.6%	1.4%	12.2%	49.3%
Isochrysis sp. (17,500 cells/L)	Mean	7,243	7, 751	673	9,246
	SD	7,918	9,002	550	9,286
	CV	109%	116%	81%	100.4%

*These two species are combined because neither UMD or ANS separated them during analysis.

Table F-3. Dominant Species in Eel Pond Samples (Average of Triplicate Counts in cells/L +SD)

Species		Concentration (cells/L ±SD and CV)		
		UMD		ANS
Volume settled (mL)		1000 mL	50 mL	50 mL
Analysis method		Sedgwick-Rafter	Inverted	Inverted
Analysis location		UMD	WHOI	ANS
Microflagellates	Mean	488,297	693,462	185,765
	SD	21,091	26,057	28,087
	CV	4.30	3.8	15.1
Cryptomonas	Mean	215,026	388,877	151,564
	SD	45,394	10.642	49,043
	CV	21.10	2.7	3.2
Guinardia delicatula	Mean	33,996	53,381	32,532
	SD	7,084	13,014	3,312
	CV	20.80	24	10.20
Chaetoceros costatum	Mean	67,043	38,227	73,869
	SD	10,947	20,378	11,986
	CV	16.30	53	16.2
Skeletonema costatum	Mean	7,624	20,247	17,629
	SD	3,674	9,516	6,518
	CV	48.20	47.0	36.9
Leptocylindrus sp.	Mean	6,899	12,143	10,500
	SD	4,892	8,759	4,020
	CV	70.90	72	38

*These two species are combined because neither UMD or ANS separated them during analysis.

Table F-4. Dominant Species in Massachusetts Bay Samples (cells/L)

	URI			ANS
Sample	Dominant Species	1000 ml settled; counted using SR slide at UMD	1000 ml settled; counted using inverted slide at WHOI	50 ml settled; counted using inverted slide at ANS
A	Microflagellates	722,034	365,195	737,858
	Gymnodinium sp.	4,915	1,671	56,073
	Cryptomonas sp >10 um	0	0	50,890
	Cryptomonas sp <10 um	0	0	38,167
	TOTAL	730,846	370,597	905,550
B	Microflagellates	689,032	472,306	1,841,596
	Guinardia delicatula	175,355	347,185	570,152
	Leptocylindrus danicus	133,548	372,750	0
	Cryptomonas sp.	726	32,689	285,076
	Gymnodinium sp.	806	4509	157,852
	TOTAL	1,004,005	938, 908	3,377,712
C	Microflagellates	740, 645	342,446	1,268,384
	Calycomonas wulfii	0	0	48, 784
	Cryptomonas sp. >10 um	0	0	35, 340
	Cryptomonas sp. < 10 um	0	0	44, 293
	Gymnodinium sp.	323	780	43, 350
	TOTAL	742,574	349,240	1,474,465
D	Microflagellates	617,333	not counted	1,865,988
	Guinardia delicatula	10, 500	not counted	37,696
	Skeletomena costatum	917	not counted	480, 624
	Cryptomonas sp. >10 um	0	not counted	285, 861
	Cryptomonas sp. <10 um	0	not counted	202, 676
	TOTAL	647,167		3,579,171
E	Microflagellates	321,563	317,480	536,624
	Leptocylindrus danicus	28,211	4,329	4,712
	Guinardia delicatula	1,707	231,616	1,178
	Rhizoselenia fragilissima	0	0	88, 350
	Skeletonema costatum	0	0	70, 680
	Gymnodinium sp.	650	1,443	70, 680
	TOTAL	356,114	558,950	1,018,149

Appendix G

Right Whale Guidance Protocol for Vesssels Operated/Contracted by the Commonwealth of Massachusetts (21 November 1997)

Guidance Protocol on the Interaction with Whales
Specifically Northern Right Whales for Vessels Operated/Contracted by the
Commonwealth of Massachusetts

Introduction

The northern right whale is the most endangered large whale in the world. In the western north Atlantic the population is estimated to be about 300 animals. Massachusetts coastal waters are part of the range of the northern right whale and Cape Cod Bay has been designated a critical habitat for the whale under the federal Endangered Species Act because of its high use by the species in the late winter and early spring for feeding. The Great South Channel, east of Cape Cod, has also been designated critical habitat because of its importance to the right whale as a feeding area. It has been determined that the most significant human induced causes of mortality are ship strike and entanglements in fishing gear.

Purpose

The purpose of this protocol is to give guidance to the vessels owned by the Commonwealth and those operating under contract to the Commonwealth as to proper operational procedures if the vessels should encounter whales - *i.e.* sighting and reporting procedures, and entanglement and carcass reporting protocol.

Applicability

This protocol will apply to all vessels owned by the Commonwealth of Massachusetts and/or contracted out by the Commonwealth of Massachusetts.

Geographic Scope/Operational Scope

This protocol applies to all applicable vessels operating in or adjacent to Commonwealth waters. When vessels are operating in the designated critical habitat areas (Cape Cod Bay or the Great South Channel) heightened operation is applicable, especially during the late winter and spring when the right whales are expected to be located in these areas.

Sightings of Right Whales

The Executive Office of Environmental Affairs and the National Marine Fisheries Service is interested in receiving reports from individuals who observe right whales during vessel operations. Reports should be made to the National Marine Fisheries Service Clearinghouse. Patricia Gerrior, NMFS Right Whale Early Warning System Coordinator, who manages the Clearinghouse and her numbers are 508-495-2264 (work), 508-495-2393 (fax) and pager 508-585-8473. Please report your name, agency and phone numbers at which you can be contacted. The vessel's name, the date, time and location of the sighting, the numbers of whales sighted and any other comments that may be of importance. If a camera or video camera is available please take some photographs. These photographs should be provided to Pat Gerrior or Dan McKiernan, Massachusetts Division of Marine Fisheries. They will in turn send copies to the New England Aquarium for comparison to the Right Whale Photo Identification Catalog. **Please remember that Massachusetts has Right Whale Conservation Regulations (322 CMR 12:00) which establishes a 500 yard buffer zone surrounding a right whale. Vessels shall depart immediately from any buffer zone created by the surfacing of a right whale.**

Physical Contact with a Whale

If a vessel owned by the Commonwealth of Massachusetts or under contract with the Commonwealth of Massachusetts comes into physical contact with any whale it should be noted in the vessel's logbook. The vessel's logbook should include the time and location of the incident; weather and sea conditions; vessel speed; the species of whale struck if known; the nature of any injures to crew, and/or the whale, and/or damage to the vessel. Also record the name of any other vessels in the area that may have witnessed the incident or can provide information about circumstances. A copy of the vessel's log for the entire trip should be submitted to the Director of the Division of Marine Fisheries, the Director of the Division of Law Enforcement, the Secretary of Environmental Affairs and the National Marine Fisheries Service, Northeast Region in Gloucester.

If after hitting the whale, the animal is incapacitated or appears to have life threatening injuries and the vessel is safe and secure, immediately call the Center for Coastal Studies, entanglement hotline at 800-900-3622 or via their pager at 508-803-0204 and the Massachusetts Environmental Police Communications Center at 800-632-8075 or 617-727-6398. Stay with the whale until the Coast Guard or Center for Coastal Studies arrives on scene.

Entanglements

If the vessel come upon or entangles a right whale immediately notify the Center for Coastal Studies' entanglement hotline at 800-900-3622 or via their pager at 508-803-0204 and the Massachusetts Environmental Police Communications Center at 800-632-8075 or 617-727-6398. Do not attempt to remove any debris from the whale, stay on station with the whale or follow at a safe distance. As relocating an entangled whale can be extremely difficult, staying on station or following the animal is very important. However, if following the whale is not possible contact, the Coast Guard and/or the Center for Coastal Studies and note the last direction the animal was heading and any other pertinent information that would assist in relocating the whale.

Stranded Whales

For a stranded right whale please notify the Stranding Network immediately call Connie Merigo or Howard Krum, New England Aquarium, Central Wharf, Boston, MA 02110. The standing Network's hotline is 617-973-5247 (pager) or as a second resort call 617-973-5246/6551.

QUICK REFERENCE

Sightings & Photographs

Patricia Gerrior, NMFS Right Whale Early Warning System Coordinator, manages the Clearinghouse and her numbers are 508-495-2264 (work), 508-495-2393 (fax) and pager 508-585-8473

Photographs

Dan McKiernan, Massachusetts Division of Marine Fisheries, 19th Floor, 100 Cambridge Street, Boston, MA 02202. 617-727-3193 ext. 369.

Entanglements or Injured whales

Center for Coastal Studies, entanglement hotline at 800-900-3622 or pager at 508-803-0204

Massachusetts Environmental Police Communications Center at 800-632-8075 or 617-727-6398.

Stranded Animals

The standing Network's hotline is 617-973-5247 (pager) or as a second resort call 617-973-5246/6551.